

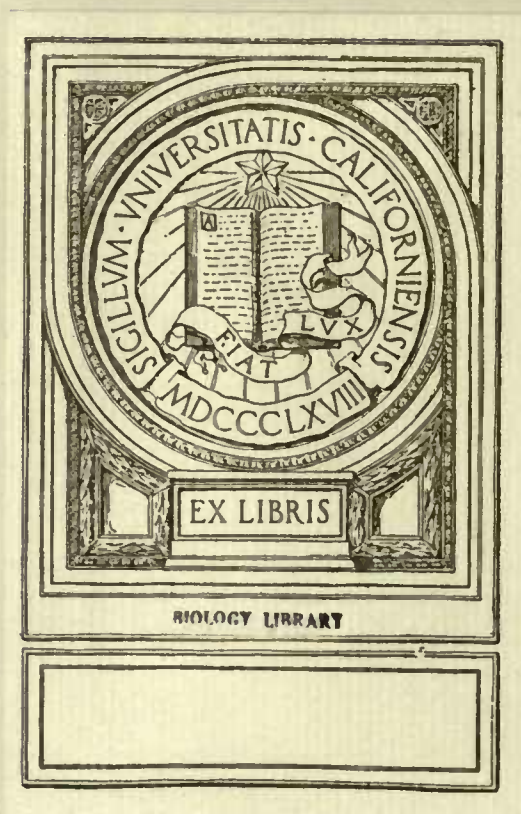
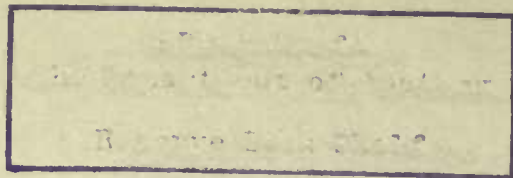
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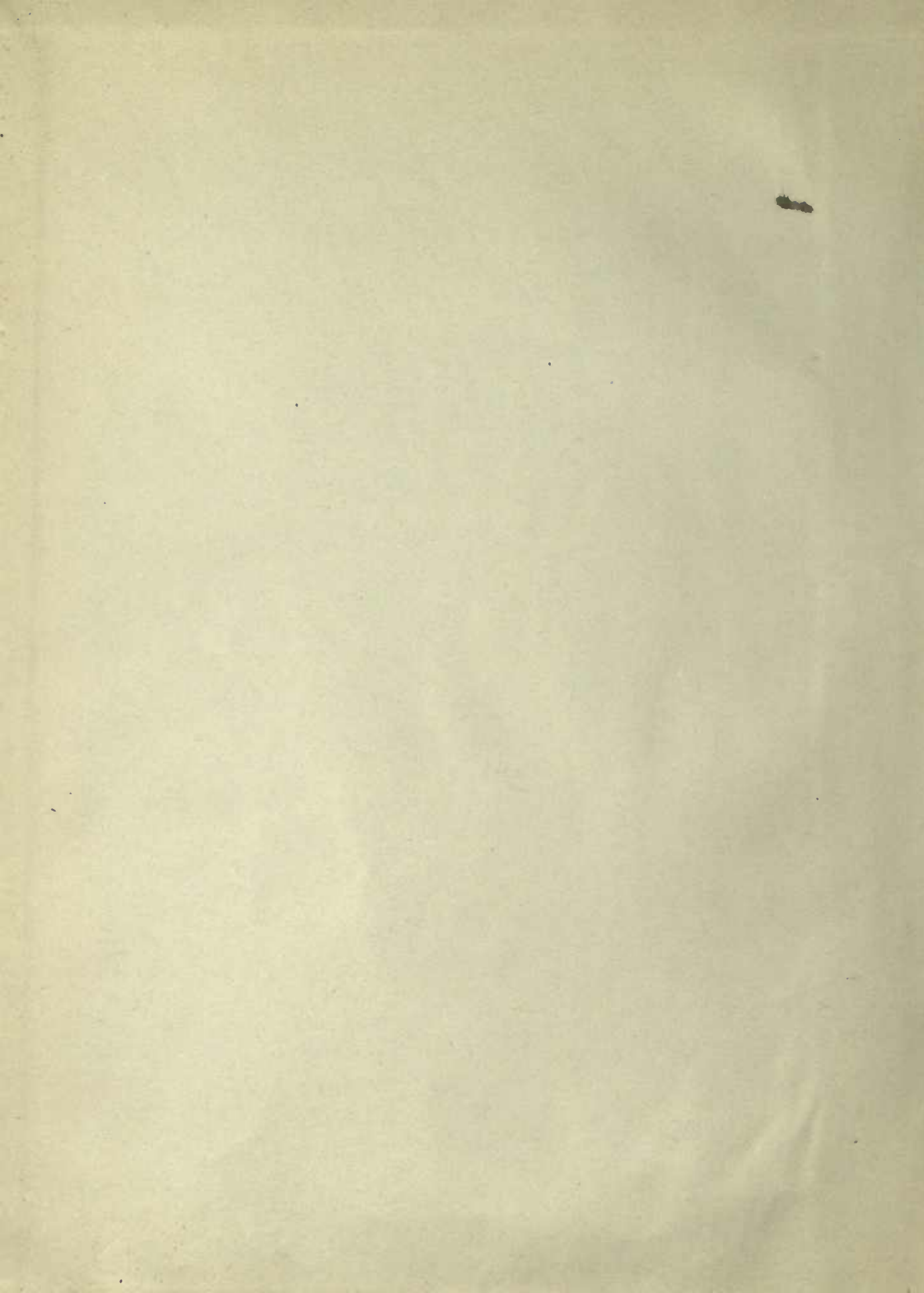
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THE STRUCTURE OF THE GLANDS OF BRUNNER

BY

ROBERT RUSSELL BENSLEY

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THE STRUCTURE OF THE GLANDS OF BRUNNER

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THE STRUCTURE OF THE GLANDS OF BRUNNER

ROBERT RUSSELL BENSLEY

I. INTRODUCTION

THE appearance recently of the papers of Castellani (1898) and Rock (1899), dealing with the structure of the glands of Brunner, is a sign of renewed interest in a group of glands which has baffled both the physiologist and the anatomist. The efforts of the former have been largely directed toward investigating the presence or absence of digestive ferments in the succus entericus or in artificial extracts of the gland. Their investigations, undertaken with this object in view, have not yielded uniform results, although the recent studies of Glässner (1902) confirm the observations of Grützner (1872) that the glands of Brunner contain a proteolytic ferment.

Wepfer (1679), who discovered the glands in 1679, described their arrangement in the duodenum, and observed that when macerated in water they liberated an abundant amount of mucus. Eight years later the glands were described more fully by Brunner, who regarded them as a sort of secondary pancreas. The incorrectness of this view was shown by Claude Bernard (1856) and Middeldorpf (1846), both of whom pointed out that the secretion of the glands of Brunner differed from that of the pancreas.

Budge and Krolow (1870) found that the extract of the glands of Brunner would transform starch into sugar, would dissolve fibrin in acid solution, but would not act on coagulated albumen nor on fats.

Grützner (1872), following up his observations on the proteolytic ferments of the pyloric glands, which he, in common with Heidenhain (1870) and Ebstein (1870), regarded as pepsin-forming glands composed of chief cells like those of the fundus glands, found that he could obtain by extraction of the glands of Brunner with 0.1 per cent. hydrochloric acid a solution which would rapidly digest fibrin in acid solutions.

Similar positive results as to the existence of a proteolytic ferment were obtained by Gachet and Pachon (1898), who introduced cylinders of coagulated albumen into the isolated duodenum after tying the pancreatic duct.

Recently Glässner (1902) has extended to the study of the ferments of the glands of Brunner the methods which he had already employed with success to separate the various ferments of the gastric mucous membrane. The extracts which he obtained, after taking all possible precautions to exclude the glands of Lieberkühn from the material extracted, and to destroy adherent pepsin and trypsin, were inactive with respect to starch, cane sugar, and fats. On the other hand, they digested fibrin, serum albumen, and coagulated egg albumen in solutions containing 0.2-0.3 per cent. of hydrochloric acid. Moreover, some proteolytic activity was still displayed when

the solutions were rendered neutral or slightly alkaline. The specific ferment upon which this proteolytic action depended, he identified with the pseudopepsin which he had previously extracted from the pyloric mucous membrane. It differed from pepsin in that it was not destroyed, nor its activity removed, by weak solutions of sodium carbonate, and that its action quickly led to the formation of tryptophan. From trypsin it was distinguished by its activity in acid solutions.

In view of these experiments of Glassner, Grützner, and others, it seems certain that the cells of the glands of Brunner contain a proteolytic enzyme. As yet, however, it is not known whether this is a tissue enzyme concerned in some of the various metabolic processes of the cell itself or a secreted product of the cell designed to assist in the intestine, in the transformation of the proteids of the food.

Anatomically the main points of interest, as far as the glands of Brunner are concerned, have been the form and distribution of the glands, the question as to whether they are mucous or serous glands, the changes they exhibit in different stages of physiological activity, their relationship to the pyloric glands, and their phylogeny.

The question of the form of the glands has been adequately treated by Schwalbe (1872), whose conclusions that the glands of Brunner are composed of ramifying tubules into which acini open have been confirmed with some additional details, by the studies of Maziariski (1902) and Peiser (1903), who employed the reconstruction method of Born.

Concerning the mucous or serous nature of the glands, however, there is not the same unanimity of opinion. By many authors, including Schwalbe (1872), Heidenhain (1872), Bentkowski (1876), and Piersol (1894), they have been compared with the pyloric glands which these authors regarded as similar to the chief cells of the fundus glands. Claude Bernard (1856), Sappey (1876), Renaut (1879), and Berdal (1894) regarded them as mucous glands. Renaut (1879), basing his conclusions on the study of the glands of Brunner of man, regarded them as structures differentiated for the secretion of a peculiar mucus. He compared them with the pure mucous glands of the œsophagus and bronchi, which, according to him, have the same fundamental structure as the glands of Brunner.

The same view, in a somewhat modified form, was taken by Kuczynski (1890), who studied in a number of representative mammals the staining reactions of the glandular cells by means of certain synthetic dyes, in particular victoria blue, azoblue, aniline blue, and thionine. These he found to stain the cells of the glands of Brunner of different mammals with different degrees of facility. Some cells, for example those of the pig, were refractory to all attempts to stain them. He concluded that in the latter animal the cells contained no mucin; that in others, for example the rabbit and horse, where the staining was feeble, the amount of mucin was small; and again that in the guinea pig and ox, where the cells stained strongly, they contained a large amount of mucin.

Schaffer (1891) obtained a slight violet coloration of the cells of the glands of

Brunner of man with hæmatoxylin; and concluded that the mucus which they secreted differed materially from that formed by goblet cells and by the salivary glands.

Castellant (1898) concluded that the glands of Brunner could not be regarded as mucous glands, but should rather be compared with the pyloric peptic glands. He added, however, that the differences, considerable in the rat, slight in the dog, between the glands of Brunner and the pyloric glands, led him to believe that their secretion was not identical, but that the glands of Brunner secreted a special digestive liquid.

A similar view of the nature of the Brunner's glands is taken by von Ebner (1899), who bases his conclusions as to their non-mucous nature on the failure to stain them with Mayer's mucicarmine and muchæmatein.

The lack of unanimity of opinion as to the nature of the glands of Brunner is to be traced to a number of causes. Of the first importance, in this connection, is the resemblance of the glands of Brunner to the pyloric glands of the stomach which Heidenhain (1870, 1878) and his pupils had shown, apparently conclusively, on physiological grounds, to be pepsin-forming serous glands. This conclusion, as far as the pyloric glands are concerned, has been shown to be erroneous by the writer (1896, 1898) on histological and microchemical grounds, and for chemical reasons by Glässner (1902), who has confirmed the conclusion of the writer that the glands of the pyloric region do not contain pepsin-zymogen (propepsin). A second cause which has contributed to this confusion of results is the lack of precision in our morphological criteria for distinguishing between mucous and serous cells. In the absence of specific knowledge of the chemistry of the secretion of a gland, a mucous gland in which the nucleus was spherical and the cytoplasm abundant would invariably, according to the older ideas, be interpreted as a serous gland.

The classification of glands into mucous glands and serous glands is at the best a mere makeshift. When we have decided that a gland is a serous gland, we may still know absolutely nothing about the nature of its secretion, beyond the fact that it does not contain a mucin. There are, however, a number of serous glands the secretion of which it is possible to collect and examine chemically, and which are known to be largely engaged in the secretion of digestive enzymes. To this category belong certain of the serous salivary glands, the pancreas, and the chief cells of the gastric glands. In recent years these zymogenic glands have been the subject of numerous investigations, as a result of which we now have a tolerably exact knowledge of their structure in the different phases of physiological activity. The serous glands have been investigated by Solger (1894, 1896, 1898), Erik Müller (1895), Zimmermann (1898), Garnier (1900), and others; the pancreas, by Eberth and Müller (1892), Mouret (1895), Macallum (1891, 1895, 1898), and Matthews (1899); the gastric glands, by Bensley, (1896, 1898, 1902), Zimmermann (1898), Theohari (1899), and Cade (1901).

These researches show that serous glands which are known to be zymogenic in function, whatever their source, have certain features in common, due to the presence

in the cell of the several substances antecedent to the secretion. These are, briefly, the *zymogen* granules which occupy the portion of the cell nearest the lumen, and which are easily visible in the fresh cell, and the *prozymogen*, a nucleoproteid substance probably of nuclear origin, remarkable for its staining power, located in the base of the cell. The latter substance is often unequally distributed in the basal cytoplasm, giving rise to the appearance of radial striation, to which we owe the name "basal filaments," first employed for the prozymogen of the glandula submaxillaris of man by Solger (1896).

Both of the substances mentioned above as characteristic constituents of the zymogenic cell give microchemical reactions which enable one to distinguish them with some confidence from the substances antecedent to secretion in a mucous cell. The fact that the prozymogen is a nucleoproteid enables one to employ the microchemical tests for iron and phosphorus for its identification, and no structure in any cell should be likened to the basal filaments of the serous zymogenic cell unless it does give a positive result with these tests. Furthermore, the granules of zymogen may be positively distinguished from granules of mucigen by the fact that after careful extraction with alcohol and ether in a Soxhlet apparatus to remove the lecithins, they give a strong reaction by Macallum's method, showing the presence in them of organic phosphorus as an elementary constituent.

Mucous cells, on the other hand, do not contain any basal filaments, and the feeble reaction for organic iron which they give indicates that they contain a relatively small amount of diffused prozymogen. As pointed out above, the secretion granules of mucous cells do not give any reaction for organic phosphorus.

In addition to the above characters which we can apply in distinguishing serous from mucous cells, we are able, owing to the researches of Paul Mayer (1896) on the methods of staining mucus, to obtain, with much greater certainty than before, a positive staining reaction for this substance in cells by employing the special solutions of hæmatein and carmine devised by him.

Up to the present, there has been no attempt to apply these methods to the study of the glands of Brunner. We must, therefore, compare these glands as regards the structure, staining and microchemical reactions, and the changes exhibited by them in different phases of functional activity, not only with the nearly adjacent gastric and intestinal glands, but also with the many glands from other sources which have been the subject of exact investigation.

A point on which most writers agree is the great similarity between the glands of Brunner and the pyloric glands of the stomach, although recent researches of Kuczynski (1890), Castellani (1898), and others do not confirm the conclusions of Bentkowski (1876) and Schiefferdecker (1884) that the two sorts of glands are identical.

The question of the relationship of the glands of Brunner to the pyloric glands is mainly interesting from the standpoint of the phylogenesis of the former. Although

they occur for the first time in mammals, the fact that they have been found without exception in all mammals belonging to the three main subclasses in which they have been sought, indicates that they must have appeared very early in the history of the Mammalia. The fact that the pyloric glands and the Brunner's glands have been subject throughout this long phylogenetic history to conditions which are not identical would alone be sufficient to explain slight differences in the nature of their cells, even if, as some suspect, the two sets of glands have had a common origin. Furthermore, in many groups of animals, owing to a change of food habits, the stomach has undergone important secondary adaptive modifications, resulting in the suppression of extensive areas of gastric glands, the replacement of the cylindrical epithelium by a stratified squamous epithelium, and the modification in type of the remaining glands. It is reasonable to expect that, under these extraordinary conditions, the pyloric glands would be modified, and the differences between them and Brunner's glands intensified. Clearly, it would be unfair to assume that, because the pyloric-gland cells differ from those of Brunner's glands in an animal with such a specialized stomach, they are primitively different. In other words, in discussing the question of the difference or similarity of the two sets of glands, both the long phylogenetic history of the glands and the relative visceral specialization of the animal under discussion must be given due weight.

The present memoir embodies the results of an investigation undertaken with a view of applying to the solution of the problems presented by the glands of Brunner the microchemical and staining methods employed by the writer in the study of the glandular elements of the stomach, and the more recent conception of the structure of these and other cells. With this end in view, the glands of Brunner of one marsupial and of nineteen placental species representing six orders of mammals have been submitted to a careful anatomical and histological examination. The results from a phylogenetic standpoint are somewhat disappointing, but it is hoped that they will bring us nearer to a proper conception of the morphology of the glands in question.

The material for study included the pyloric glands and glands of Brunner of the opossum, dog, cat, mink (*Lutreola*), raccoon (*Procyon*), hedgehog (*Erinaceus*), porcupine (*Erethizon*), guinea pig, ground hog (*Arctomys monax*), squirrel, rabbit, dormouse (*Muscardinus avellanarius*), muskrat (*Fiber zibethicus*), mouse, rat, deer mouse (*Peromyscus*), sheep, pig, and man.

The glands of Brunner of the opossum have been selected for the preliminary description, because the shape and structure of the stomach in this form correspond so closely to those which we have reason to believe were primitive in mammals that we may expect to find primitive conditions in the glands, and because the peculiar characteristics of the glands of Brunner in the opossum furnish, it is thought, the strongest argument which could be brought forward in favor of the theory that they are produced directly from the pyloric glands.

II. THE GLANDS OF BRUNNER OF *DIDELPHYS VIRGINIANA*

The glands of Brunner in the opossum form a narrow ring around the pyloric orifice of the stomach. In a preparation of the stomach and duodenum, cut open along the line of attachment of the hepatogastric and hepatoduodenal ligaments, one may see in the mucous membrane at the gastro-duodenal junction with a low magnification, a number of minute funnel-shaped depressions, forming two or three irregular rows more or less parallel with the line of junction of the stomach and intestine, and at distances of two to three millimeters apart. On examination in sections these prove to be the openings of tubular depressions of the mucous membrane into which the glands of Brunner open. These depressions are not, however, in the strict sense, the ducts of the glands, but rather evaginations of the mucous membrane as a whole, because, in the inner portions of them, villi project from the wall into the cavity, and small intestinal glands occur, beneath which a continuation of the longitudinal fibers of the lamina muscularis mucosæ may be seen. These intestinal elements may be traced to a short distance below the level of the lamina muscularis mucosæ of the stomach and intestine, where the depression opens into a cavity of considerable size, around which the lobules of the glands of Brunner are clustered, and into which the ducts of the latter open. If the stomach and intestine, and after ligation of the latter some inches below the pylorus, be distended with the fixing fluid, under sufficient pressure to overcome the resistance of the pyloric sphincter and to dilate the opening to a width of about 1.5 cm., one sees at the gastro-duodenal junction, instead of the depression referred to above, a number of rounded patches which are smooth and devoid of villi. In sections of such a preparation, the appearance represented in Plate XIX, Fig. 1 is observed; the lobules of the glands of Brunner are clustered around a place where the ordinary elements of the intestinal mucous membrane are lacking and only an epithelium is to be seen. Through large openings in this epithelium empty the ducts of the glands of Brunner. The first row of these defects in the intestinal mucous membrane occurs at or near the gastro-duodenal junction, that is to say, the first row of patches into which the glands of Brunner open may be continuous with the mucous membrane of the stomach, or may be separated from the latter by a minute interval, about 0.1 mm. in width, in which villi, intestinal glands, etc., may be found. In the former case, the glands of Brunner have the appearance of being a continuation of the pyloric glands; in the latter, they appear to be entirely separated from them.

The extent of the glands of Brunner in a longitudinal direction is about 6.7 mm., beginning about 1.2 mm. above the point where the gastric and duodenal epithelium meet, and extending a distance of 5 mm. to 5.5 mm. into the tela submucosa of the duodenum. They form a series of angular lobules, some entirely separate, others aggregated into larger or smaller lobes.

The glands are of a branched acinotubular type. The smaller lobules have as a rule a single tubular duct which opens on the surface of one of the depressions above

described. This duct penetrates to the center of the lobule giving off radial tubular branches which subdivide repeatedly until an exceedingly complex structure is produced. All tubules below the main duct give off at frequent intervals short tubules which are regarded by Maziarski (1902) as acini. The cells lining all these branches of the main duct, and indeed those of a large portion of the duct itself, are of precisely the same character.

The tubules are surrounded and supported by the collagenic connective tissue of the tela submucosa, in which may be seen near the epithelium of the defect small strands of smooth muscle fiber which represent the remains of the lamina muscularis mucosæ. In the tissue between the glands numerous mast cells may be seen, some lying free in the connective tissue, others closely applied to the outer surface of the glandular epithelial cells.

The defect of the tunica mucosa, into which the ducts of the glands open, is covered by a single layer of epithelial cells. At the edge of the patch these consist of the usual epithelial elements of the intestine, namely goblet cells, cylindrical epithelial cells with basal cuticula, and granule cells of Paneth. Epithelium of this character, however, extends over a very small portion of the extreme margin of the patch and is broken by only a few ducts. The greater portion of the area is covered by cylindrical epithelial cells of the mucigenous type. The shape and dimensions of these surface epithelial cells vary within wide limits, according to the tension and shape, that is, convexity or concavity, of the surface upon which they rest. On relaxed portions of the surface, they are very high and narrow, 17–27 μ in height by 5–7 μ in width. If, however, the tissue is fixed under tension, they appear short and wide and the position and shape of the nucleus are similarly modified.

At first sight the resemblance of the epithelium to that of the stomach is remarkable, but on close examination it is found that this resemblance does not amount to identity, although the differences are not of great importance. As in the gastric epithelium, the distal portion of the cell is occupied by a mass of secretion forming a distinct theca. The proximal portion of the cell is occupied by finely reticular cytoplasm and contains an oval nucleus. The latter may be slightly flattened where it comes into contact with the mass of secretion. The differences between these cells and those of the gastric epithelium are particularly emphasized in specimens stained in iron alum hæmatoxylin, in muchæmatin, or in mucicarmine. In the former stain the theca of the gastric epithelial cell appears of a slightly grayish color and homogeneous. The masses of mucin (granules?) which fill the theca are so closely aggregated that there is but little cytoplasm left in the free ends of the cell to retain the stain. At the base of the theca only, can be made out a delicate network, which extends from the cytoplasm between the theca and the nucleus for a short distance into the mass of secretion. In the epithelial cells of the Brunnerian area, on the other hand, the theca is subdivided by delicate strands of cytoplasm forming a network in the meshes of which the granules of mucin are lodged. Moreover, the theca is subdivided into proximal and

distal portions by incomplete bands of cytoplasm, stretching across it, parallel with the free border of the cell. The meaning of these facts will be discussed later. In the meantime it is merely intended to point out that the theca of the epithelial cells under discussion contains a larger proportion of cytoplasmic elements, as distinct from stored-up secretion, than that of the epithelium of the stomach. A further point of difference is to be seen in the size of the theca, which presents a remarkable uniformity in the gastric epithelium, but is very variable in the epithelium of the defects.

Toward the bottom of the gastric foveolæ, however, cells are found which agree very closely in structure with the cells of the defects, and these, as has been frequently pointed out, by Bizzozero (1893) and others, are connected with the cells of the surface by a gradual transition. A similar transition may be seen at the gastro-duodenal junction, where the epithelium of one of the defects happens to be continuous with the gastric epithelium. *We may therefore conclude that the epithelium of the defects is gastric epithelium not so highly differentiated as that of the surface of the stomach.*

The ducts which open on the defects are lined for a greater or less portion of their course by epithelium of the type described above, except that the cells are as a rule shorter and wider than on the free surface. At variable distances from the opening of the duct, there is transition, sometimes abrupt, sometimes gradual, to the glandular epithelium which lines all the numerous side and terminal branches of the gland.

The two animals from which the material was obtained exhibited different physiological phases of the gland; in one the cells were completely filled with secretion, in the other only partly so. A transverse section of two tubules from the latter animal is represented in Plate XX, Fig. 2. Each tubule is composed of somewhat rectangular cells, very similar in general characters, but with some differences of detail, surrounding a central cavity. The lumen varies in width from 4μ to 16μ , the largest diameter being usually found in the main branches of the duct and in those terminal tubules (acini) which lie at the margin of a lobule. In each glandular cell a number of distinct zones may be made out. Beginning at the outside of the tubule and proceeding toward the lumen, there may be distinguished, first, a narrow zone of cytoplasm, of a delicate reticular structure, stretching across the base of the cell and containing the somewhat irregular nucleus. On the distal side of the nucleus a clear zone with coarse reticular structure may be observed, then a narrow band of finely reticular cytoplasm, and finally a second coarsely reticular clear zone, bordering the lumen. The meshes of the two clear zones obviously contain the stored-up secretion of the cells, which is divided into a proximal and a distal mass by the transverse bridge of cytoplasm. This reciprocal arrangement of the cytoplasm and its product is deserving of some emphasis because of the fact that it occurs with surprising constancy in corresponding phases of secretion in mucous cells from the most varied sources, *e. g.*, salivary, palatine, oesophageal, and tracheal glands, the cardiac and pyloric gland cells of the stomach, and the neck chief cells of the fundus glands. Zimmermann (1898) observed this structure in the cells of the human glands of Brunner, but did not attempt an interpreta-

tion. Kolossow (1898), by means of his osmic-acid reduction method, obtained similar pictures in the salivary glands, and recently Maximow (1901) has demonstrated it by thionin staining in the mucous salivary glands. The writer has repeatedly called attention to this phenomenon in the mucous-secreting cells of the stomach. A character which reappears with such constancy in similar cells from so many different sources must have a very important significance.

The network which is visible in the clear zones of these cells is neither a true cytoplasmic reticulum (spongioplasma) nor an alveolar structure (*Wabenstruktur*), but is probably a derivative of the latter. In sections stained intensely in iron alum hæmatoxylin it is possible to follow the threads of the network by focusing. In such preparations it is seen that, in a great many cases, they are not fibers, as would appear at first sight, but thin laminae which intersect and join to form the visible network. The spaces of this network are, however, everywhere continuous with one another. Often, however, the proximal mass of secretion near the nucleus exhibits a true alveolar structure, the spaces being rounded cavities containing reserve secretion, separated completely from one another by the continuous cytoplasm of the zone. It is easily conceivable that the latter structure has given rise to the former by the increase in size and partial coalescence of the small secretion spaces. Usually there is a very obvious difference in the amount of residual cytoplasm contained in the proximal and in the distal clear zones respectively, the proximal zone exhibiting smaller spaces and larger cytoplasmic trabeculae than the distal zone.

The cytoplasm of these cells does not contain basal filaments, but the presence of a small amount of cytoplasmic nucleoproteid is indicated by the feeble but positive reactions for iron obtained by Macallum's methods.

In order to study under the best conditions the reciprocal relations of the cytoplasm and secretion, it was necessary to have an intense stain of the masses of secretion, leaving the cytoplasm unstained. The various synthetic dyes which were tried did not yield very satisfactory results, as, although a positive result was obtained with thionin, toluidin blue, and methyl blue, the stain was not sufficiently selective to permit of accurate definition of the cytoplasm and secretion respectively. By means of P. Mayer's muchæmatein the writer obtained a very intense and satisfactory stain of the secretion by transferring thin sections cut in paraffin, from benzole to absolute alcohol, and then to the stain, but was unsuccessful with similar sections fastened to the slide. It was subsequently found that, by gradually increasing the strength of the solution without altering the relative proportions of its solid constituents, a solution was obtained which could be used for staining sections fastened to the slide, with certainty of speedy and satisfactory results. The procedure is as follows: The stain consists of hæmatein, 1 g., aluminium chloride, 0.5 g., 70 per cent. alcohol, 100 c.c. The hæmatein and chloride are rubbed up together in a mortar, then mixed with the alcohol, the whole being allowed to stand a week to insure perfect ripeness of the solution. During this week the solution deepens in color, and its staining power for mucin

increases daily. The sections fastened to the slide by the water method are treated with benzole and absolute alcohol. The slide is then flooded with the staining solution, placed on the stage of the microscope, and watched until the intense blue color appears in the cells. The sections are then rapidly washed in 95 per cent. alcohol, dehydrated, cleared, and mounted in xylol balsam. Washing in water extracts the stain, but if the sections are first washed in 70 per cent. alcohol, then in lime water, the stain is fixed, and subsequent washing in water affects it but slowly. Similarly effective results may be obtained with mucicarmine, if the strong stock solution of Mayer be employed instead of the diluted solution. Mucicarmine can be depended on only if the solution is freshly prepared; the old solutions do not give satisfactory results. The stain obtained with mucicarmine is very stable, is not affected by washing in water, and may be used for subsequent contrast staining. Very excellent double stains, remarkably rich in detail, are obtained by staining in iron alum hæmatoxylin, followed by mucicarmine.

Muchæmatein, prepared and applied in the way described above, gives an intense blue color to the contents of the goblet cells and of the clear portion of the glandular epithelial cells of Brunner. Old solutions stain the granules of the mast cells and, if the solutions are acid, as Harris has pointed out, the coarser elastic fibers. Cytoplasm remains unstained. Cells stained in this way exhibit the exact reverse of the structure described above, and illustrated in Fig. 2. The clear zones of the cell appear filled with deeply stained secretion, the rest of the cell colorless.

Considerable interest attaches to the mode of aggregation of the secretion in the cell, and in this connection some results have been obtained which afford a simple explanation of the discordant results on the mucous salivary glands. The writer was at first considerably puzzled by the fact that in some of his preparations the secretion appeared in the form of distinct granules, in others in the form of a continuous coarse meshed network. It was speedily found, however, that if water were excluded from the operations of the technique, the granular condition was always obtained, whereas if water were introduced at any stage, the reticular appearance was obtained. For example, sections stained without fastening to the slide, or after cutting in celloidin, by simply transferring from strong alcohol to the stain, then back to alcohol, gave granules; sections passed through water, or fastened to the slide by the water method and heat, gave a network. The obvious inference was that the secretion was stored in the cells in the form of fine granules or droplets which had not been altered chemically by the fixing agents, and which on treatment with water promptly went into complete or partial solution, to be again precipitated by the stain in reticular form. It is a well-known fact that mucins outside the cell precipitate frequently in the form of a coarse network.

We may now return to a description of the cell after staining with muchæmatein in such a way as to preserve the granules of secretion. Such a preparation is illustrated in Plate XX, Fig. 3. In each cell there are seen to be two masses of

granules corresponding to the two clear zones of the cells of the preceding figure. The granules in the distal mass bordering on the lumen are very closely aggregated, very small, and somewhat angular in outline. Those of the proximal mass are less closely packed, often somewhat scattered, smaller and more rounded.

If we now combine the results of the two methods of staining, we may conceive the clear secretion-filled zones of the fixed cell to be composed of two elements, strands, threads, and delicate laminae of cytoplasm, forming a network, in the meshes of which are minute granules of secretion, the latter separated from one another, sometimes by the threads of cytoplasm, more often by clear spaces. This appearance may be interpreted in one of two ways. In the living cell the granules of secretion must be separated from one another by a continuous substance of some kind. This continuous separating substance may be either the cytoplasm or a third substance of a more watery nature, filling the interstices of the cell between the particles of secretion and the cytoplasm. In the former case the strands of cytoplasm seen in the dead cell would represent merely the contracted precipitates produced in the continuous cytoplasm by the fixing reagents; in the latter case they would represent the actual distribution of the cytoplasm in the living cell. The latter interpretation seems the more probable for a number of reasons. In the first place, it suggests a possible explanation of the capacity of the cell to vary the respective constituents of its secretion in response to specific stimuli, the possibility of which has been clearly demonstrated by the work of Pawlow (1898) and his pupils on the stomach, and by that of Malloisel (1902) on the submaxillary gland. In the second place, one frequently sees granules with minute threads of substance stainable in muchæmatein projecting from their surfaces suggesting that there is an intermediate clear substance (the hyaline substance of Langley) in which portions of the secretion of the cell exist in complete solution. A third reason, perhaps a stronger one than either of the two foregoing, is that one finds the secretion in the form of droplets or granules in the theca of goblet cells from some sources, in which strong staining in iron hæmatoxylin shows the presence of only faint, delicate threads of cytoplasm, or of none at all.

A glandular tubule from a section fastened to the slide by the usual water method and stained in muchæmatein is represented in Plate XXI, Fig. 8. The granules of secretion have disappeared and have given place to the coarse network usually seen in mucous cells. The division of the accumulated secretion into an inner and an outer mass may still be recognized in some of the cells, although the bridge of protoplasm separating these is less obvious in the midst of the deeply stained secretion. This network again is not of an alveolar character, as the laminae and trabeculae which form it are perforated and interrupted in such a way that the clear spaces throughout the mass communicate with one another.

The blue-stained network observed in muchæmatein preparations is a precipitation product, and must not be confused with the network visible in the clear zones of cells stained by the iron-hæmatoxylin method. The mucinoid material is doubtless precipi-

tated in part on the cytoplasmic meshes, but not wholly so, because the network obtained in muchæmatein is formed by much larger, coarser meshes than that seen in the iron hæmatoxylin preparation. Moreover, one may completely remove the substance stainable in muchæmatein by treatment of the sections for several hours with a dilute solution of barium hydroxide. Subsequent staining in iron hæmatoxylin shows that the cytoplasmic network in the clear zones of the cell has undergone no change.

We may now consider the changes exhibited by the cell while passing from the intermediate state described in the foregoing paragraph to the fully loaded conditions. As already pointed out, one may find in the same tubule cells in the different secretory conditions, and by comparing cells from different tubules an idea may be obtained of their secretory phases. The majority of the cells in Fig. 2 are in a condition intermediate between the loaded and the discharged states. The completely loaded stage is represented in Plate XX, Fig. 4, drawn from a tubule of a gland of Brunner of a second opossum, stained with iron hæmatoxylin. In this preparation the cell presents a swollen aspect and exhibits throughout a coarse meshwork of cytoplasm inclosing clear secretion spaces. There is no indication of a subdivision of the contained secretion into a proximal and distal mass, the transverse bridge of protoplasm seen in most of the cells of Fig. 2 being here represented only by a slight thickening of some of the cytoplasmic trabeculæ in the middle of the cell. The cytoplasm at the base of the cell, which was of considerable extent in Fig. 2, is here reduced to a minimum, and the nucleus has in many cells taken on the crescentic form so characteristic of mucous cells. The tubules of the gland in this individual were in general larger and the lumina narrower than in those from the first animal. An idea of the way in which this secretion-loaded cell is derived from those of the type illustrated in Fig. 2 may be gained by studying a large number of tubules from the same animal stained in iron hæmatoxylin and in muchæmatein. It has been pointed out that in preparations stained with muchæmatein without coming in contact with water the secretion appears in the form of rounded granules arranged in two more or less distinct groups, and that the granules of the proximal mass are frequently smaller and less crowded than those of the distal mass. The changes in the cell in the act of storing up secretion seem to be going on more actively in this inner mass of granules which increases progressively in density and extent by increase in the size of the granules and by addition of new granules. Correlated with this increase in amount and number of the droplets of secretion is a diminution of the amount of cytoplasm which takes place simultaneously in the basal cytoplasm, in that of the transverse bridge, and in that of the intergranular trabeculæ. The result is that the transverse bridge of cytoplasm presently disappears and the two masses of secretion become continuous. At the same time the amount of secretion in the distal zone is probably increasing, although this is less easily made out. A comparison of the cells in the two extreme conditions stained in iron hæmatoxylin shows that the cytoplasmic network of the distal zone is composed of larger meshes in the fully loaded cell.

A feature of some interest is the relation of the granules of secretion, as seen in muchæmatein preparations, to the nucleus of the cell. Frequently only one of the two masses above described may be seen, namely that which corresponds to the proximal mass, the edge of the cell along the lumen, in this case being cytoplasmic in nature. The obvious interpretation of this fact is that at the last period of activity this cell has thrown out all of its reserve secretion and that, while this has been going on, the cytoplasm has been increasing in amount and new granules have been forming in the proximal segment of the cell between the nucleus and the mass of old secretion. In the majority of cells, however, only a portion of the reserve secretion is so discharged and new granules are deposited alongside of the old ones in the interior of the cell.

The formation of new secretion in mucin-forming cells in close proximity to the nucleus has been observed by Krause (1895) in the cells of the retrolingual gland of *Erinaceus*, and more recently has been demonstrated by Maximow (1901) in the cells of the retrolingual gland of the dog. The meaning of this phenomenon is not clear, for although Carlier (1899) has demonstrated in the large mucous cells of the gastric glands of the newt, morphological changes of the nucleus in the different phases of secretion identical with those exhibited by the pepsin-forming cells, yet microchemical study does not reveal in mucous cells, as it does in many serous cells, the presence in the cytoplasm in large amount of an undoubted product of nuclear activity (prozymogen, ergastoplasm of Cade and Garnier). It is possible that the transformation of the substances received by the cell into mucin is accomplished by the agency of an enzyme formed in the nucleus. A further possibility is the effect of the presence, in relation with the proximal mass of secretion of the canals of Holmgren's trophospongium, although I have not yet been able to demonstrate such canals in the cells of the glands of Brunner.

If one compares the foregoing description of the cells of the glands of Brunner with the account of the cells of the retrolingual gland of the dog, recently published by Maximow (1901), he cannot fail to be struck by the extraordinary resemblance, extending even to the minutest details, between these two kinds of glands. In fact, if we leave out of account the serous tubules and cells of the retrolingual gland, the description would apply equally well to both. In the case of the glands of Brunner, it is not possible to collect the secretion as it flows from the gland and examine it chemically, but there are many reasons for supposing it to consist, like that of the retrolingual gland, largely of mucin. These reasons are briefly: (1) the strong resemblance in structure and physiological phases to cells known to be engaged in the secretion of mucin, *e. g.*, glandula sublingualis of the dog; (2) the stain, obtained in strong muchæmatein and mucicarmine, in which the protoplasm of the cylindrical, intestinal epithelium cells and the granules of the cells of Paneth remain colorless; the feeble metachromatic stain occasionally obtained in thionin; and (3) the solubility in weak alkaline solutions. The latter fact I have established by using muchæmatein as an indicator. I found that if sections attached to the slide were treated with a 5

per cent. solution of potassium carbonate, or a saturated solution of barium hydroxide, the substance in the cells of the glands of Brunner slowly dissolved. The various stages of this slow solution could be determined by treating sections from time to time with strong muchæmatein. The procedure is as follows: A number of sections fastened to slides by the water method are freed from paraffin, passed through alcohol to water, placed in a Coplin jar containing a quantity of the solution to be tested and the whole placed in an incubator at 37° C. From time to time slides are removed from the solution, carefully washed in water, and in dilute acetic acid (0.1 per cent.) if the solution used was alkaline, again washed in water, transferred to alcohol, stained in muchæmatein, cleared and mounted in balsam. In this way the steps of the reaction, if any, could be accurately followed. I found that the contents of the glands of Brunner placed in a solution of barium hydroxide saturated at room temperature disappeared in two hours; that of the pyloric gland cells, of the gastric epithelial cells, and of the goblet cells required ten to twenty-four hours for complete solution. The granules of the Paneth cells were unaffected. The mucin from all the above cells was unaffected by treatment with 5 per cent. solution of hydrochloric acid for twenty-four hours at 37° C. or by peptic digestion for the same length of time. The difference in the solubility of the mucin from the several sources—Brunner's cells, gastric epithelium, goblet cells, etc.—may be due to the unequal action of the fixing fluid (alcohol bichromate sublimate), but, on the other hand, may indicate a chemical difference in the nature of the mucins from these several sources.

The cells of the pyloric glands in the opossum resemble the cells of the glands of Brunner very closely. The differences which may be observed are of secondary importance. The tubules of the pyloric glands and the cells composing them are distinctly smaller than those of the glands of Brunner. The internal structure of the cells is, however, almost identical. The most obvious differences between the pyloric glands and the glands of Brunner consist in the great number of cells of Stöhr visible in the former and in the greater ease with which the contained secretion may be stained with muchæmatein. The mucin of the pyloric glands may be stained by a weaker solution of muchæmatein, in which the glands of Brunner remain colorless.

III. THE GLANDS OF BRUNNER OF THE CARNIVORA

Next to the opossum in order of simplicity, as regards the structure of the glands of Brunner and their relation to the pyloric glands, come the genera of the placental orders, Carnivora and Insectivora. The structure and extent of the glands of Brunner in the cat and dog have been the subjects of numerous researches and are well known. The facts as they present themselves in these animals are the foundations for most of our knowledge, both physiological and histological, concerning the glands of Brunner.

From the standpoint of specialization, the glands of Brunner of the cat and dog present an advance on the condition found in the opossum, inasmuch as the ducts,

instead of opening in groups on a special area of the mucous membrane from which glands of Lieberkühn and villi are absent, penetrate between the former, traverse the whole thickness of the mucous membrane, and open independently, on the surface, between the bases of the intestinal villi. The epithelium at the mouth of these ducts presents, as Schiefferdecker (1884) has pointed out, a quite remarkable resemblance to gastric epithelium, the same differences, however, being recognizable, as have been already described for the epithelium of the ducts of the Brunnerian area of the opossum.

The resemblance of the cells of the glands of Brunner of these animals to those of the pyloric glands is a very close one—so close, indeed, that one is justified in declaring that they are identical. In the glands of Brunner of the cat the subdivision of the secretion into two masses is very obvious; in the dog, in the resting condition, the cell is so filled with secretion that such a subdivision cannot be made out, but if the cells be exhausted by stimulation with pilocarpine or by feeding, it is seen that new secretion is deposited in the neighborhood of the nucleus and forms a proximal mass. The essentially similar changes in the pyloric-gland cells under experimental conditions of secretion have already been described by the writer (1898). The differences between the pyloric and Brunner's glands of these animals consist in the greater size of the constituent tubules, to a large extent due to the expansion of the lumen, and in the absence of the granular cells of Stöhr from the glands of Brunner. Another difference which is particularly obvious in the cat is the greater variability of the cells of Brunner's glands in respect to the amount of mucin they contain.

In both of these animals the secretion stains readily with muchæmatein and mucicarmin, applied in the way described above. For the cat and dog, however, I have obtained the most satisfactory stain by employing the eosin-aurantia-indulin mixture of Ehrlich diluted with twenty times its volume of water. By this means the secretion of the glands of Brunner and of the pyloric glands is stained intensely blue, the rest of the tissue red or yellow. The indulin mixture discriminates between the mucin of the glands of Brunner and that of the goblet cells and of the gastric epithelium, and brings out exquisitely the transition in type of the epithelium in passing up the ducts.

In the raccoon, the glands of Brunner extend a distance of about 35 mm. into the duodenum. They form an aggregate of a number of fairly distinct elliptical lobules imbedded in a tela submucosa composed of unusually dense, close-textured, collagenic fibrous tissue. The lobules are confined to the tela submucosa except for the short distance (1.6 mm.) at the beginning of the intestine where a few groups of Brunner's glands (pyloric glands?) occur in the deeper layers of the mucosa among the somewhat dispersed fibers of the lamina muscularis mucosæ.

The lamina muscularis mucosæ is continuous, but perforated here and there by the ducts of the glands of Brunner. For some distance on each side of the sphincter pylori it is composed of longitudinal fibers only, the circular inner layer making its appearance toward the end of the glands of Brunner. From the outer surface of the

lamina muscularis mucosæ smooth muscular fibers radiate into the connective tissue of the tela submucosa, forming a more or less perfect network around the glandular lobules. In the mucous membrane the intestinal epithelium and glands begin just beyond the pyloric sphincter, there being as a rule very little intermingling of intestinal and gastric elements, although for a short distance (8 mm.) a few pyloric glands, serving as ducts for the superficial glands of Brunner, could be discerned.

The lobules are composed of branching tubules of large diameter and lined by large secretion-filled epithelial cells. Each lobule is provided with a duct, often of extraordinary size, which penetrates the lamina muscularis mucosæ, often presenting at this point an ampulla-like dilatation, extends through the mucous membrane a variable distance, and terminates in a shorter or longer gland of Lieberkühn. Rarely these ducts reach the free surface without being intercepted by a gland of Lieberkühn. A minority of the ducts open into the bottoms of the intestinal glands, directly after having passed through the lamina muscularis mucosæ. The majority extend about half the thickness of the mucous membrane, become abruptly reduced in size, and are continued to the surface by a gland of Lieberkühn. A few reach the surface without alteration in the type of epithelial cells. Whenever the ducts open into a gland of Lieberkühn, the change of epithelium is abrupt.

The cells of the glands of Brunner from the single individual available for examination resembled the secretion-filled stage of the opossum's glands. They were from 16μ to 22μ in height and filled with secretion. In iron hæmatoxylin preparations the cell exhibited a delicate network with very large meshes, the nucleus being crowded off into one of the basal angles of the cell. A peculiarity of the nuclei in these cells was that, instead of occupying the middle of the base of the cells, they were in one corner and flattened parallel to the lateral walls of the cells. Otherwise the condition was the same as in other secretion-filled mucous cells. *In strong muchæmatein solutions the cells stained intensely and showed a very coarse network of blue-stained trabeculae.*

No trace of a division of the secretion into two masses was discernible in the cells of the gland tubules, but those of the ducts exhibited a distinct transverse band of cytoplasm, so dividing the secretion.

The resemblance between the cells of the glands of Brunner and those of the pyloric glands in the raccoon is very close, the only character in which a difference could be made out being the subdivision of the secretion into two masses, which was obvious in the pyloric glands, but in the glands of Brunner visible only in the cells of the ducts.

In the mink (*Lutreola vison*), the glands of Brunner are confined to the submucosa and form a compact mass, beginning opposite the pyloric sphincter about 1 mm. beyond the point of greatest thickness of the latter, and extending a distance of about 12 mm. into the duodenum. The glands are composed of more or less rounded lobules closely packed together in the submucosæ, which they almost fill from the lamina

muscularis mucosæ to the tunica muscularis. The amount of interlobular connective tissue is relatively small. The zone begins with a number of small scattered elliptical lobules confined to the superficial portion of the tela submucosa, but rapidly increases in width and in number of lobules as the duodenum is descended. The greatest width, 0.7 mm., is reached at a distance of about 5.3 mm. from the beginning of the zone. From this point onward the layer of glands gradually diminishes in thickness until it disappears, so that the whole mass has a fusiform shape in longitudinal section.

The lamina muscularis mucosæ is continuous throughout except at the points where the ducts pass through to enter the tunica mucosa. It consists of a thin internal layer of circular fibers and a thicker external layer of smooth muscle fibers. The structure of the mucous membrane covering the Brunner's glands of the mink is unique, inasmuch as the tunica mucosa of the proximal half of this region, although on the duodenal side of the pyloric sphincter, is covered by gastric epithelium and occupied exclusively by glandulæ pyloricæ identical in structure with those which occur in the stomach itself. These pyloric glands extend into the duodenum a total distance of 8.8 mm. (measured from the summit of the sphincter), slightly beyond the point of greatest thickness of the glands of Brunner. Here they are abruptly succeeded by the intestinal epithelium and glands.

Beyond the point where this change takes place, however, for a distance of 0.3 mm. one may meet pyloric glands mingled with the glands of Lieberkühn, opening into typical foveolæ gastricæ, and may even find patches of gastric epithelium on the surface. There is no lymphatic nodule at the junction of gastric and intestinal mucosæ; both of these mucosæ in this animal are singularly poor in lymphatic tissue.

The glands of Brunner of the proximal half of the zone open directly into branches of the pyloric glands by ducts which pierce the lamina muscularis mucosæ. Beyond the point where the intestinal glands of Lieberkühn make their appearance the glands of Brunner open either into the glands of Lieberkühn or into the pyloric glands with which these are mingled. In the distal parts of the area independent ducts are rare, the glands opening almost exclusively into the bottoms of Lieberkühnian glands. A curious fact in these latter instances is that the epithelium of the proximal branches in the lobule may be lined by cylindrical and goblet cells; in other words, the glands of Lieberkühn, instead of receiving the ducts of the glands of Brunner in the tunica mucosa internal to the lamina muscularis mucosæ, perforate the latter and may subdivide into several branches which retain the typical epithelium before receiving the tubules formed of the epithelium characteristic of the glands of Brunner. We may thus have illustrated in this single animal all the various kinds of ducts between two extremes, one of these being the condition in which independent ducts lined by glandular epithelium and opening into gastric foveolæ are present; the other, that in which the branched glands of Lieberkühn penetrate the tela submucosa and receive the tubules of Brunner's glands.

The tubules of the glands of Brunner in the mink bear a very close resemblance

to those of the cat. They have, as a rule, a rather large lumen surrounded by secreting cells. The average size of the tubules is about 40μ , but some are as much as 77μ in width.

The cells vary in width from 11μ to 15μ , the two extremes being often found on opposite sides of the same tubule. The cells correspond very closely in structure to those of the opossum; indeed, Fig. 2 would illustrate equally well a typical tubule from the mink. In each cell two well-defined secretion masses may be discerned separated by a transverse band of cytoplasm. *The secretion in these cells stains strongly in muchæmatein applied as indicated above.* The amount of secretion contained in the cells, and so the amount of cytoplasm, vary respectively, in the material examined, with the distance from the sphincter pylori. In the glands near the sphincter, the cells contain a large amount of basal cytoplasm and a spherical nucleus. The proximal mass of secretion is small and stains less intensely in muchæmatein. Going down the zone, the size of the proximal mass progressively increases and encroaches on the basal cytoplasm, the nucleus at the same time becoming correspondingly compressed and crescentic and crowded to the extreme base of the cell.

If we compare the cells of the glands of Brunner of the proximal part of the zone with the cells of the pyloric glands which immediately overlie them, there appears to be an almost perfect similarity of structure. But if we compare Brunner's gland cells from the duodenal end of the zone with pyloric glands several millimeters above the pyloric sphincter, some differences of structure are apparent. In the latter instance the tubules and cells are of about the same size, but in the pyloric glands the cells are to a large extent filled with cytoplasm. The proximal mass of secretion in these upper pyloric-gland cells is scarcely visible, and the distal mass next to the lumen occupies often less than one-fifth of the whole length of the cell. The nuclei are spherical. Again in the pyloric glands as the sphincter is approached the amount of secretion in the gland cells is gradually increased. There is thus in the mink the most perfect transition between the pyloric glands, on the one hand, and the intestinal elements, on the other.

IV. THE GLANDS OF BRUNNER OF ERINACEUS

In the European hedgehog the glands of Brunner form a mass of rather large lobules, beginning at the pyloric sphincter and extending a distance of 9.3 mm. into the duodenum. The greatest thickness of the mass is exhibited at the beginning, where the lobules fill the space formed by the sudden falling off of the pyloric sphincter. Here it reaches a thickness of 1.8 mm. From this point it gradually diminishes in thickness to the end of the zone.

The glands are separated from the tunica mucosa by a lamina muscularis mucosæ composed of longitudinal fibers, which form a continuous layer except for a short distance just at the beginning of the duodenum, where the fibers are somewhat inter-

rupted by the ducts of the proximal lobules of the glands of Brunner. At this point the pyloric glands and the glands of Brunner appear in places to be continuous. The dispersed fibers of the lamina muscularis mucosæ radiate out among the lobules of the glands, forming a partial investment for them. At other points a duct which is passing through the muscularis mucosæ may give off a few gland tubules which are thus located among the muscular fibers of the layer.

The ducts of the glands of Brunner in the hedgehog are independent of the glands of Lieberkühn, between which they pass to open on the free surface. A cluster of ducts from the proximal lobules occurs just at the junction of the intestinal and the gastric mucous membrane, some opening between the gastric epithelium on one side and the first villus on the other; the rest, between the bases of the first short villi. In the rest of the zone, the ducts occur at scattered intervals and pass through the mucous membrane without branching or showing a change in type of the epithelium. The glands are composed of richly branching tubules radiating from a central duct in each lobule. The terminal branches are frequently short acinus-like tubules which in fixed preparations, however, seldom show a terminal dilatation.

The tubules are composed of large cells of rectangular or pyramidal shape, surrounding a small lumen. The latter is larger in the tubules which are nearer the main ducts and largest in the ducts themselves. The cells in the specimen from which the descriptions are taken vary in length from $16\ \mu$ to $21\ \mu$, the average being $18\ \mu$. The cells in hæmatoxylin sections are clear, transparent, and filled with secretion, between the droplets of which a delicate network may be made out. In the material at the writer's disposal, very few of the cells of the gland tubules exhibit a subdivision of the secretion into two masses. The nuclei are irregular and located at the bases of the cells. In the ducts, on the other hand, the amount of residual protoplasm gradually increases as the surface is approached, and the nucleus expands to an oval shape with the long axis coinciding with that of the cell. In the stronger muchæmatein the contained secretion stains deeply.

The cells of the pyloric glands are very similar to those of the glands of Brunner, although they are somewhat smaller (average length $15\ \mu$) and contain less mucin. Cells of Stöhr are frequent in the pyloric glands, but absent from the glands of Brunner. The secretion in the cells of the pyloric gland, like that of the glands of Brunner, stains with stronger muchæmatein.

V. THE GLANDS OF BRUNNER OF THE RODENTIA

Among the rodents it is only in the suborder Myomorpha that the glands of Brunner are confined to the proximal section of the duodenum, between the pylorus and the opening of the ductus communis choledochus. In the rabbit, belonging to the Duplicidentata, the American porcupine, belonging to the Hystricomorpha, and the squirrel, marmot, and gopher (Oppel) belonging to the Sciuromorpha, they extend far beyond the opening of the bile duct.

In the rabbit, according to Kuczynski (1890), these glands occur in the duodenum as far as the opening of the ductus pancreaticus, a distance of 30 cm.

The glands in the rabbit make their appearance in the tela submucosa at the point where the intestinal epithelium begins. For a distance of about three and one-half centimeters they form two groups, one located in the tunica mucosa, the other in the tela submucosa. These groups are incompletely separated from one another by the lamina muscularis mucosæ, which is interrupted here and there, to permit of communication between the superficial and the deep glands, and the passage of ducts from the latter to the surface. Throughout the rest of the duodenum the glands are for the most part confined to the tela submucosa, although here and there a duct may give off a small group of branches in the tunica mucosa before reaching the submucous group.

The ducts of the superficial lobules of the glands open directly into the glands of Lieberkühn; those belonging to the submucous group preserve their characteristic epithelium for a short distance after passing through the lamina muscularis mucosæ, but are in the majority of cases continued to the free surface by a gland of Lieberkühn. There are, however, a few independent ducts in which the epithelium retains the type characteristic of the glands of Brunner as far as the free surface.

The glands of Brunner of the rabbit are unique in structure, inasmuch as they contain two kinds of cells. This fact was discovered by Schwalbe (1872) who described the more deeply staining elements as independent glands, having the same structure as the pancreas. Schwalbe's observations have been confirmed by Dekhuyzen (1888) and Kuczynski (1890). The latter author showed that the deeply staining elements did not, as Schwalbe thought, form independent glands, but occurred along with the clear cells in the same tubules. Dekhuyzen came to the conclusion that the two kinds of cells represented different functional stages of the same element, and claimed to have seen intermediate types. Castellant (1898) gives an excellent description of the deeply staining cells, in which he observed the small refractive granules of the distal zone and the striation (basal filaments) of the basal zone. He takes exception to the conclusion of Dekhuyzen on the grounds that he could not discover any intermediate stages between the two kinds of cells, and that deeply staining cells are altogether absent in the glands at the beginning of the duodenum. Castellant does not come to any definite conclusion as to the relative specificity of the two kinds of cells, but suggests an interesting comparison between the glands of Brunner of the rabbit and the mixed glands of the trachea.

My own observations confirm the conclusion of Kuczynski that the deeply staining cells occur in the same tubules as the clear cells. The glands of the rabbit are of the compound acinotubular type, the main tubules and some of the terminal acini being composed of clear cells; many of the terminal tubules or acini, of deeply staining cells. The deeply staining cells may occur (*contra* Castellant) in the glands of Brunner at the very beginning of the duodenum.

The transparent tubules are formed by large, clear cuboidal cells surrounding a

lumen 16μ to 20μ in width. The clear cells of the glands of Brunner of the rabbit are mucous cells, very similar in type to those of the animals already described. A number of tubules formed by cells of this type is represented in Plate XXI, Fig. 5. The body of the cell presents a coarsely reticular structure, the meshes of which are filled with the reserve secretion of the cell. This secretion may be stained by the use of the stronger muchæmatein, or of mucicarmine, or of Mann's methyl blue-eosin in which it stains blue, or of the eosin-aurantia-indulin mixture recommended by the writer for staining the secretion of the pyloric glands. The appearance of the secretion when so stained differs according to the mode of fixation and subsequent treatment of the tissue. In material fixed in aqueous solutions of corrosive sublimate, the secretion presents the reticular appearance characteristic of mucous cells. The same remark is true of material fixed, without previous removal of the tunica muscularis, in the alcoholic sublimate-bichromate mixture. If, however, the muscular coat of the intestine is removed, and the organ then fixed in the last-mentioned mixture, so as to permit the sublimate and bichromate to come at once into contact with the cells, the secretion is found to be in the form of distinct granules which are, when so fixed, more resistant to the action of water and therefore more permanent in preparations than the corresponding structures in the cells of the opossum. According to the phase of functional activity the secretion is subdivided more or less completely into proximal and distal masses. In Fig. 5, which represents the fully loaded condition, traces of the transverse band of cytoplasm separating the two masses are still visible in most of the cells. The basal cytoplasm is small in amount and contains no basal filaments.

Sections of the fresh tubule examined in blood serum or in salt solution appear perfectly transparent, the granules of secretion corresponding so closely in refractive index to the surrounding medium that they remain invisible.

In material fixed in absolute alcohol and tested by Macallum's microchemical method for the detection of organic compounds of iron, a very faint positive reaction is obtained in the cytoplasm around the nucleus. By Macallum's modification of Lilienfeld and Monti's method for the microchemical detection of phosphorus, the cytoplasm gives a reaction no stronger than that obtained in the cytoplasm of the cylindrical cells of the surface epithelium. The secretion masses remain colorless in this test.

Both in the fresh tissue and in stained sections, the dark tubules present appearances totally different from those just described. In the fresh material examined in blood serum the dark tubules exhibit two well-marked zones. The outer zone is perfectly clear and transparent, no structures whatever being visible in it. The inner zone, on the contrary, is occupied by large numbers of closely packed, minute, highly refractive granules. The appearance of such a fresh tubule is shown in Plate XXI, Fig. 7. The resemblance to a fresh pancreatic tubule or to an active acinus of a serous gland is striking.

In the stained preparations these tubules are distinguished from the mucous tubules by their remarkable staining capacity. The lumen is so small as to be scarcely

visible in many tubules. The cells are of about the same height as the mucous cells, but as a rule narrower and more triangular in outline.

The two zones visible in the living cell are even more obvious in the stained preparations. The basal clear zone of the living cell is distinguished by its great capacity for staining. In hæmatoxylin, carmine, toluidin blue, safranin, and many other nuclear dyes, the basal zone of the cell stains intensely. The substance on which this capacity for staining depends is not evenly distributed in the basal zone, but, as Castellant (1898) has pointed out, gives to the zone an indistinct radially striated appearance (Plate XXI, Fig. 6).

The inner zone of the cell, unless special precautions have been taken to preserve and stain the granules, appears clear with a fine alveolar structure, the spaces corresponding to the granules of the fresh cell, the framework to the cytoplasm separating them.

By Macallum's microchemical test for organic iron, a very intense positive reaction is obtained in the deeply staining substance of the basal or proximal zone. A similarly intense reaction is obtained in this substance, after extraction of the lecithin by alcohol and ether in a Soxhlet apparatus, by the use of Macallum's microchemical reaction for the detection of organic phosphorus. By the latter method a positive result also is obtained in the granules of the inner distal zone.

The microchemical reactions indicate that the substance of the basal zone on which the capacity for basic dyes depends is a nucleo-albumin or nucleoproteid substance, probably the latter, similar to that found in the basal zones of cells from various serous glands, as, for example, the pancreas, the chief cell of the body of the gastric gland, and the cells of the serous glands of the gustatory area of the tongue, the serous cells of the human submaxillary, etc. For this substance, the writer has employed the term "prozymogen," first used by Macallum (1891), to designate a substance which he found in the pancreatic cell by the use of safranin. He afterward (1895) employed the name to designate the iron-holding organic compound of the basal zone of the pancreatic cell, which he identified with the safraninophilous substance of his earlier studies. In 1895 Mouret described the reciprocal relation between the deeply staining filaments of the pancreatic cell previously described by Eberth and Müller (1892), Platner (1889), Macallum (1891), and others, and the zymogen granules, and applied independently of Macallum and without being aware of his work, the name "prezymogen" to the substance of the basal zone. In 1896 Solger described, without attempting an interpretation of them, the deeply staining filaments in the basal zone of the serous cells of the human submaxillary gland, and Erik Müller (1895) observed similar structures in the submaxillary gland of the guinea pig. In the same year I (1896) published a preliminary account of my researches on the gastric glands of vertebrates, in which it was shown by microchemical tests that the so-called basal filaments of serous cells owed their affinity for basic dyes to the fact that they contained a chemical substance similar to chromatin

which was identified as Macallum's prozymogen. In this study it was shown that the prozymogen of the base of the cell increases and diminishes in amount *pari passu* with the diminution and increase respectively of the zymogen granules, and it was therefore regarded as an antecedent substance of the latter. The occurrence of similar substances in the basal zones of the serous cells of the glands of von Ebner and of the oesophageal glands of the frog was also described, and it was suggested that the basal filaments of Solger and Erik Müller were of a similar nature.

Since the publication of this paper, the presence of so-called basal filaments has been demonstrated in a host of serous cells from various sources by Zimmermann (1898), Garnier (1900), Theohari (1899), Cade (1901), and others. The three last mentioned accept in a modified form the interpretation of the writer that the so-called basal filaments contain the antecedent substance of the zymogen granules—a conception which was also adopted by Solger (1899) for his basal filaments of the submaxillary gland of man.

It is clear from the foregoing that the dark-staining cells of the rabbit's glands of Brunner agree with serous cells of many other glands in containing large quantities of prozymogen in their outer zones and in containing in their inner zones granules, visible on the fresh cell, of a phosphorus-holding substance, presumably some sort of zymogen. This fact should speedily lead to a chemical examination of the glands of Brunner from this animal to determine the nature of the secretion of these cells. On anatomical and microchemical grounds, there would appear to be a stronger possibility of a positive result in the search for important digestive ferments in the glands of Brunner of this animal than in those of any other mammal.

The conclusion that the dark cells of the glands of Brunner of the rabbit are serous cells is further borne out by the negative evidence afforded by staining in muchæmatein and mucicarmin, in which the secretion granules of these cells remain absolutely colorless.

The facts in connection with the glands of Brunner of the rabbit may be summed up as follows: The glands of Brunner of the rabbit are mixed glands (well compared by Castellant to the mixed glands of the trachea) composed of mucous portions, the cells of which stain strongly in muchæmatein, mucicarmin, etc.; and serous portions, which do not stain in these solutions, but on the contrary possess a basal zone with indistinct radial striation containing a large amount of prozymogen, which may be demonstrated by the microchemical reactions for iron and phosphorus, and an apical zone in which minute granules, presumably of zymogen, are to be seen. These two types of cell are morphologically and chemically distinct from one another, and no intermediate types are to be found.

The pyloric glands of the rabbit are very similar to those of the glands of Brunner, but the former, as Dekhuyzen (1888) pointed out, are, as a rule, smaller and contain more cytoplasm and less secretion than the latter. However, by selecting an animal that has been fasting for some time, pyloric glands may be obtained the cells of

which are indistinguishable by cytological characters from those of the glands of Brunner. In the pyloric glands many cells of Stöhr may be seen. In the glands of Brunner I have failed to find any of these elements.

In the American porcupine the duodenum presents at its beginning a flask-shaped dilatation about 3 cm. in length, into which the common bile duct opens. The glands of Brunner are not, however, confined to this, but extend, as in the rabbit, for a considerable distance into the duodenum. The piece of duodenum available for this examination was 12 cm. in length and throughout contained glands of Brunner. In no part of this area do the glands reach any considerable development. They form a relatively thin layer in the superficial portion of the submucosa, composed of rather small lobules often consisting of a few tubules only. Some tubules may also be seen in the tunica mucosa above the lamina muscularis mucosæ. The glands begin immediately distal to the pyloric sphincter. At this point a well-defined group is present in the mucosa and forms the direct continuation of the pyloric glands of the stomach, although they open either directly, or by means of a gland of Lieberkühn between the villi. Separating these from the small lobules in the submucosa there is a well-defined lamina muscularis mucosæ composed of a thick outer longitudinal stratum and a thin incomplete inner circular layer. In the rest of the region the lamina muscularis mucosæ is thin, composed almost wholly of longitudinal fibers and much interrupted by the passage of ducts. The ducts of the glands of Brunner after passing through the lamina muscularis mucosæ open either directly into the bottoms of the glands of Lieberkühn or ascend as independent ducts for a short distance and open into the sides of the glands of Lieberkühn shortly before reaching the surface. In the latter case the duct is usually joined by a number of tubules which are located in the mucosa. There are also in the mucosa small groups of Brunner's glands which open independently into the sides or bottoms of the glands of Lieberkühn without being connected with those located in the submucosa or with their ducts.

The glandular tubules are formed of cells of a rectangular shape 8μ to 17μ in height, the average being about 13μ . The cells show great uniformity in structure, notwithstanding differences in size. The nucleus has a distinct oxyphile nucleolus, is situated at the base of the cell, and is oval in shape, but is in some cases somewhat flattened or irregular from compression. The body of the cell is transparent in hæmatoxylin preparations. There is but little cytoplasm at the base of the cell, but the secretion is, as in most other mammals, distinctly divided into two masses by a bridge of cytoplasm similar to that illustrated for the opossum in Fig. 2. The proximal mass also, as in the opossum, exhibits a coarser cytoplasmic network than the distal mass. In strong muchæmatein a coarse network, composed of deeply blue-stained trabeculæ, is seen.

The few pyloric glands which are found on the intestinal side of the summit of the pyloric sphincter are exactly similar in all structural details to the glands of Brunner. On the gastric side, however, marked differences are visible which are particularly

apparent in sections stained in muchæmatein. In such preparations the pyloric-gland cells exhibit a very narrow blue-stained margin along the lumen. The proximal mass of secretion in the neighborhood of the nucleus is either wholly absent or only indicated. In the iron hæmatoxylin sections the rest of the cell is found to be filled with cytoplasm free from secretion. The nucleus is full, spherical or oval in outline, rich in chromatin, and separated by a distinct interval from the base of the cell. In size the cell is about the same as the Brunner's gland-cell. The pyloric glands of the porcupine contain numerous cells of Stöhr.

In the guinea pig (*Cavia cobaya*) the glands of Brunner are feebly developed, although they extend a considerable distance into the duodenum, according to Kuczynski (1890) 10 cm. Even at its thickest part, near the sphincter pylori, the layer may be not more than 0.25 mm. in thickness. For a distance of about 7 mm. it forms a fairly continuous layer of thin lobules, but beyond this point the lobules become very small and occur at increasingly greater intervals. Each lobule is composed of a cluster of branching tubules connected by a short duct with the bottom of a gland of Lieberkühn.

The tubules are composed of cuboidal to cylindrical or prismatic cells, varying in height from 9.5μ in the small flattened tubules of the distal lobules to 14μ to 18μ in the proximal lobules. The nuclei of these cells are irregularly crescentic in shape and are located in the extreme outer ends of the cells. The body of the cell exhibits the usual transparent reticular appearance when examined in preparations stained in iron hæmatoxylin. There is usually in the middle of the cell a slight condensation of the cytoplasm, a suggestion of the subdivision of the secretion into two masses. In some of the cells, particularly in those of the ducts near the points where they are about to open into the glands of Lieberkühn, and in those forming the tubules of the small distal lobules, a very obvious band of this condensed cytoplasm may stretch across the cell. In the latter case the cytoplasmic trabeculæ which separate the granules of the proximal mass are coarser in texture and form smaller meshes than those of the distal zone. These facts indicate the probability that the mechanism of secretion in the glands of Brunner of the guinea pig is similar to that in the corresponding glands of the opossum and many other mammals.

The cells of the pyloric glands immediately adjacent to the pylorus are exactly similar to those of the glands of Brunner. The glands more remote from the pylorus are formed of wedge-shaped cells 12.8μ to 14.3μ in height, surrounding an extremely small lumen. The nuclei of these cells are spherical or oval in shape and located in the base of the cell. The secretion, which stains readily in stronger muchæmatein, occupies a considerable portion of the cell inclosed by the meshes of a cytoplasmic reticulum. In many cells, however, there is a proximal continuous cytoplasmic layer around the nucleus in which may be seen in iron-hæmatoxylin preparations large, coarse, rounded granules, concerning the interpretation of which the writer is in doubt. Perhaps they represent an antecedent substance of the mucin. This is the only

instance in which the writer has seen in the pyloric glands of mammals large granules which are unstainable by mucin stains and which might be confused with zymogen granules. They do not occur in the apical zone of the cell in the midst of the mass of secretion, nor may they be seen in the cells of the glands of Brunner. Similar large granules occur in the mucous cells of the pyloric glands of *Plethodon erythronotus*.

In the ground hog (*Arctomys monax*) the glands of Brunner are direct continuations of the pyloric glands. They begin at the summit of the pyloric sphincter and extend a considerable distance into the intestine—in one specimen throughout the whole piece, 9.5 cm. in length, available for examination. At no point does the glandular layer reach any considerable thickness, the maximum, measured in one specimen, being 0.3 mm. At the beginning of the intestine, the lamina muscularis mucosæ is absent and the glands located in the tunica mucosa and in the tela submucosa respectively form a continuous mass. Farther down the lamina muscularis mucosæ is represented by an interrupted band of longitudinal fibers which subdivides the glands of Brunner into two groups, one located in the submucosa tissue, the other in the mucous membrane. At the lower end of the piece the lobules are small, few and scattered, and are entirely confined to the tela submucosa.

A few of the ducts at the beginning of the zone reach the surface between the villi. Most of them, however, and all of those of the distal portion of the zone, are connected with the surface by means of a gland of Lieberkühn.

The glands are formed of rather large tubules, $31\ \mu$ to $61\ \mu$ in width, of which the lumen forms approximately one-third. The tubules branch much less freely than in the other genera already described.

The tubules are formed of large, transparent, secretion-filled cells $12.7\ \mu$ to $19.5\ \mu$ in length. The nuclei of these cells are elliptical, in some cells crescentic, in outline, and placed transversely in the proximal ends of the cells. The transparent bodies of the cells exhibit a coarse meshed reticulum composed of cytoplasmic trabeculæ, the meshes of which are filled with the secretion. The latter forms a single continuous mass in each cell, but the similarity in secretory mechanism between this cell and the corresponding cells of the opossum is shown by the differences in character of the cytoplasmic trabeculæ of the proximal and distal portions of the cell. In the proximal portion, the trabeculæ are coarser, with smaller meshes, so that the cell when stained by a strong cytoplasmic stain exhibits two zones. At the junction of these two zones there is a slight concentration of the cytoplasm, probably corresponding to the transverse band which in the intermediate stage of the opossum's cells separates the two masses of secretion.

In the ground hog the cells of the pyloric glands are similar to those of the glands of Brunner. The secretion of both stains readily in the stronger muchæmatein and in mucicarmine. In Mann's methyl blue-eosin the secretion of the pyloric glands, as well as that of the cells of the independent ducts of the glands of Brunner, stains more intensely than that of the glands of Brunner.

As in the marmot, so in the squirrel and gopher (*Spermophilus citillus*) do the glands of Brunner, according to Oppel (1897) extend a considerable distance beyond the point where the common bile duct enters the duodenum.

In the red squirrel (*Sciurus hudsonicus*) I have traced the glands for a distance of 24.6 mm. from the pyloric sphincter. In the specimen from which this measurement was taken the glands, for a distance of 8.3 mm., formed a compact mass, in which separate lobules could not be made out, completely filling the tela submucosa. For a further distance of 6.45 mm. the lobules were distinct, each lobule corresponding to a group of ducts. For the last ten millimeters only scattered small lobules were found, each consisting of a few acini, opening into the bottom of a gland of Lieberkühn.

The glands of Brunner in the squirrel make their appearance at the point opposite the pyloric sphincter where the intestinal epithelium succeeds the gastric epithelium. At this point they are located both in the tunica mucosa and the tela submucosa, the former group being a direct continuation of the pyloric glands. The lamina muscularis mucosæ of the intestine at this point is very imperfect, so that the lobules of the two groups are continuous, the fibers of the muscular lamina being dispersed among the lobules of the glands of Brunner. Beyond the first five millimeters the lobules which are seen in the tunica mucosa are less numerous and are mainly ducts which have subdivided before penetrating into the submucosa.

In one specimen examined by the writer, comprising 10 mm. of the duodenum, the ducts of the glands of Brunner were independent of the glands of Lieberkühn and were lined throughout by cells similar to those of the glandular tubules. In a second specimen, in which the pyloric glands extended a distance of 1 mm. into the duodenum, the proximal group of glands of Brunner opened, together with the pyloric glands, by means of the gastric foveolæ. Beyond the point where the first gland of Lieberkühn made its appearance independent ducts were rare, the ducts opening into the glands of Lieberkühn either as soon as they entered the tunica mucosa, or at various levels between that point and the middle of the layer. The scattered lobules of the lower 10 mm. of the zone opened exclusively into the bottoms of the glands of Lieberkühn.

The cells composing the glands of Brunner in the squirrel are subcylindrical in shape and from 15.9μ to 17.2μ in height. The large spherical or elliptical nucleus, placed in the proximal half of the cell, is surrounded by a considerable basal layer of cytoplasm. The inner half of the cell is clear and coarsely reticular. The subdivision of this distal clear segment of the cell into two secondary clear zones by a band of cytoplasm stretching across the cell from side to side is very obvious in many of the cells of the glands of Brunner of the squirrel.

The secretion in the glands of Brunner is very easily stained, even over-ripe solutions of hæmatoxylin giving successful results. In stronger muchæmatein it stains intensely, and in such preparations presents the appearance of a coarse-meshed net-

work. In such muchæmatein preparations the relations of the two masses of secretion may be readily studied. In some of the cells the two masses may be quite distinct; in others there is a deeply staining mass on the free border of the cell, another in the interior, and a faintly staining neck of secretion connecting them. Sometimes the proximal mass is subdivided into two secondary masses, one on each side of the nucleus. In all cases the proximal mass of secretion is closely applied to the surface of the nucleus.

None of the cells in my material was so filled with secretion that the nucleus was flattened by compression.

The cells of the pyloric glands for a short distance (about 6 mm.) above the sphincter are exactly like the glands of Brunner, but exhibit a progressive transition to the type of gland cell which is characteristic of the rest of the pyloric area. In the latter the cells in form and size, as well as in the position and shape of their nuclei, are very similar to the cells of the glands of Brunner. The differences between the cells from the two sources are concerned only with the amount of secretion in the cell. In the pyloric-gland cells the basal cytoplasm extends to very near the free border. The mass of secretion along the free border (distal mass) is much narrower than in the glands of Brunner. The proximal mass in the interior of the cell is represented by a few scattered granules or is absent altogether. The same differences thus occur between the glands of Brunner and the pyloric glands of the stomach as have been already remarked in the mink and the porcupine.

The Myomorpha are represented in the writer's material by the mouse, white rat, dormouse (*Muscardinus avellanarius*), deer mouse (*Peromyscus*), and muskrat (*Fiber zibethicus*). All of these are distinguished from the forms already discussed in this paper by the specialized condition of the stomach. This specialization is carried to the highest degree in the muskrat and deer mouse, in which the gastric glands have disappeared from the whole stomach with the exception of a circular area of fundus glands at the summit of the curvatura major, the pyloric glands being represented only by a very narrow zone, in *Fiber* a few millimeters in width, around the pyloric orifice. In *Mus* the specialization is not so great as in *Fiber* and *Peromyscus*, the whole right division of the stomach being occupied by gastric glands. In the dormouse the stomach is also specialized, but as the specialization is of a different kind, the comparison with the other genera as regards its degree cannot be made. In the dormouse this specialization consists in the formation of a bulb-like dilatation containing fundus glands, at the point where the œsophagus joins the stomach.

In all these genera the glands of Brunner are of small extent and present obvious differences from the pyloric glands, more particularly in the muskrat, deer mouse, and dormouse.

In the muskrat, the glands begin abruptly on the distal side of the pyloric sphincter as a thick mass completely filling the tela submucosa of the intestine and extending into the submucosa underneath the small pyloric-gland area; most of which

is on the distal side of the thickest portion of the sphincter. The lamina muscularis mucosæ is not present as a distinct layer throughout the zone, but is represented by bands of smooth muscle running in various directions among the glands. The glands of Brunner are so closely packed in the submucosa and deeper layers of the mucosa that there is very little division into distinct lobules. The ducts open into the bottoms or sides of the glands of Lieberkühn.

The glands have the usual shape and structure, *i. e.*, are composed of repeatedly branching tubules terminating in elongated pear-shaped acini or short tubules. The whole system of ducts and branches is formed of similar cells.

A transverse section of a tubule of a Brunner's gland from the muskrat is shown, highly magnified, in Plate XXII, Fig. 9. The large lumen is surrounded by somewhat cylindrical cells 13μ to 17μ in height, filled with secretion. The secretion is more or less obviously divided into a narrow distal and a larger proximal mass by a band of cytoplasm. The nucleus is flattened or crescentic, and located in the extreme outer end of the cell.

In Plate XXII, Fig. 10 are shown two glands from the pyloric region of the stomach drawn at the same magnification. The differences between this figure and the preceding one are so apparent that they scarcely require comment. The cell of the pyloric gland is much smaller, measuring 10.5μ to 11.3μ . The secretion is confined to a narrow band along the free border, and the rest of the cell is occupied by reticular cytoplasm containing an oval nucleus rich in chromatin. Several cells of Stöhr may be seen.

The secretion contained in the cells of the glands of Brunner and of the pyloric glands stains readily in strong muchæmatein; that of the glands of Brunner staining the deeper color. That this difference in the intensity of the staining does not mean a greater concentration of the mucin in the two cells is indicated by the result obtained in sections stained with Mann's methyl blue-eosin. In this the secretion contained in the cells of the pyloric glands stains deep blue, that of the glands of Brunner pale blue.

In the deer mouse the extent of the glands of Brunner, measured in one specimen, was 2.6 mm. The cells of the glands differed from those of the muskrat in the specimen examined in that the proximal mass of secretion was less compact than the distal mass, and the segment of the cell in which it was located contained relatively more cytoplasm. There was in addition a narrow basal layer of cytoplasm containing the slightly flattened oval nucleus. The cells of the pyloric glands differed from those of Brunner's glands in this animal in much the same way as in the muskrat. The pyloric-gland cells were very small, had a relatively large oval nucleus, and contained but a small amount of stored-up secretion. The secretion of both kinds of cells stained readily in stronger muchæmatein.

In the dormouse examined (*Muscardinus avellanarius*) the glands extended a distance of 3.5 mm. into the intestine. Throughout this region the lamina muscularis mucosæ was represented only by scattered fibers. The glands formed a thin continu-

ous layer in the submucosa, except at the end of the zone, where the glands terminated as scattered small lobules. The ducts emptied into the glands of Lieberkühn.

The differences between the cells of the glands of Brunner and those of the pyloric glands were greater in this animal than in any other mammal examined. The former cells were large, transparent, 15–17 μ in height, completely filled with secretion without any indication of subdivision into two masses. The nucleus was crescentic and placed in the base of the cell. The cells of the pyloric glands were small, 9 μ in height, contained very little secretion, and possessed a relatively large oval nucleus. The secretion contained in both kinds of cells stained readily in muchæmatein and mucicarmine.

In the white rat the glands of Brunner begin abruptly as a mass of considerable thickness occupying the space formed by the sudden falling off in thickness of the muscular coat on the dorsal side of the sphincter pylori. According to Kuczynski (1890), they extend into the duodenum a distance of 4.2 to 9 mm. In one specimen measured by the writer the extent was 5.5 mm. The greatest thickness of the mass is exhibited at the very beginning of the zone, where they reach a thickness of 1 mm. From this point onward the zone rapidly diminishes in thickness and, beyond a point 3 mm. to 3.5 mm. from the sphincter, is only represented by scattered small lobules. Throughout the zone the lamina muscularis mucosæ is defective. The glands discharge into the glands of Lieberkühn.

The cells of the glands of Brunner of the rat have recently been the subject of a careful study by Castellant. This author finds that the glands of Brunner of the rat present no anatomical relation to the pyloric glands and that the study of their fine structure places them still farther apart. He describes the cells as follows: "*Leurs cellules sécrétantes, de forme pyramidale présentent un contenu divisé en deux zones; l'une basale, granuleuse, où se trouve le noyau; l'autre, apicale qui reste claire, quelque soit le liquide fixateur employé, alcool, liquide de Flemming, acide osmique.*" He found that staining with hæmatoxylin after Flemming's fluid did not color the clear zone of the glands of Brunner. Mayer's mucicarmine also, in Castellant's hands, gave negative results, but thionin staining after treatment with acetic acid gave a faint reddish color which he thought might be interpreted as revealing the presence of a little mucin.

Castellant also studied the mechanism of secretion in the cells of the glands of Brunner of the rat in preparations fixed in Flemming's fluid. He found that for the first two hours of digestion these cells increase in size, the apical clear zone increases in size, and the basal granular zone is diminished in amount. From the third hour of digestion onward he found a progressive reduction in the amount of secretion in the cell to the end of the seventh hour, when the cell is almost wholly granular and contains little secretion.

Castellant does not specify the exact nature of the differences which he found between the pyloric gland cells and those of Brunner's glands, but it may be inferred that the clear subdivision of the cell into two zones in the glands of Brunner is one of them.

The use of stronger muchæmatein which stains intensely the secretion of the cells both of the glands of Brunner and of the pyloric glands of the rat has enabled me to extend somewhat the description given by Castellant. As the latter points out, the distal (apical) zone of the cell in the rat is remarkable for the extreme tenuity of the fibers of the cytoplasmic network which it contains. It does not, however, contain the whole of the secretion of the cell. In sections stained in muchæmatein it may be seen that even cells in the resting condition, in which a large proximal mass of cytoplasm is visible, may contain small granules of stainable secretion, in the portion of this cytoplasm between the nucleus and the deeply stained secretion-filled distal zone of the cell. In some of the cells these granules have so increased in number that the places occupied by them are recognizable in hæmatoxylin-eosin preparations as clear spaces in this portion of the cell. This appearance marks the transition phase from the resting condition of Castellant's observations to the more loaded condition of the third hour of digestion, and corresponds exactly to what I (1898) have found to be the case under similar experimental conditions in the pyloric glands of the cat. The explanation is that the cell when it passes from the resting to the active phase begins to transform rapidly into mucigen the reserve material contained in its basal cytoplasm, the product of this transformation making its appearance in the space between the old secretion and the nucleus. During the first hours of digestion this transformation of antecedent substance into mucigen goes on more rapidly than either the secretion of the mucin from the cell or the repair of the basal cytoplasm from which it is formed. The result is the increase in reserve secretion in the cell. A cell in this condition of maximum loading presents three distinct zones; a narrow basal zone of protoplasm containing the now slightly flattened nucleus; then the proximal mass of secretion, subdivided by coarse trabeculæ of cytoplasm; then the third zone, with which it is continuous and into which it passes by gradual transition. The third zone contains the distal mass of secretion along the free border of the cell and is remarkable for the extreme delicacy of the cytoplasmic threads which penetrate it.

The cells of the glands of Brunner of the rat may therefore present one of three conditions according to the phase of activity. Either there is one narrow mass of secretion along the free border; or there are two distinct masses of secretion, one along the free border and one in the interior of the cell; or, finally, there is a single continuous mass of secretion which shows evidences in the structure of the included cytoplasmic trabeculæ of its having been produced by the fusion of two masses originally distinct.

The cells of the pyloric glands of the rat differ from those of the glands of Brunner in the same way as do the corresponding structures in the muskrat, dormouse, and deer mouse. The cells of the glands of Brunner are from 13.3μ to 17.2μ , and those of the pyloric glands from 8.9μ to 10.8μ in height, although an occasional cell may reach a height of 13μ . The averages are 15.7μ for the glands of Brunner, and 9μ for the pyloric glands. The differences of size are less obvious when both glands are in the fully loaded condition.

The secretion of the glands of the pyloric region forms a narrow band along the lumen which is much more compact in structure than the secretion in the glands of Brunner, and like the contents of the theca in the gastric epithelium cell, retains some color in sections stained in iron hæmatoxylin, in which the secretion of the Brunner's glands are colorless. It also stains more intensely blue in Mann's methyl blue-eosin. The basal cytoplasm is more granular in appearance, and the oval nucleus is larger and richer in chromatin, than in the cells of the glands of Brunner.

The glands of Brunner and the pyloric glands of the mouse correspond so closely in structure and staining reactions with those of the rat, that they do not call for special description.

VI. THE GLANDS OF BRUNNER OF THE ARTIODACTYLA

The topography of the glands of Brunner of the Ungulates has been recently studied by Hock (1899). In the horse he found the glands to extend the enormous distance of seven meters into the intestine. In the pig and the sheep—two of the four genera of the Artiodactyla examined by him—they were also of considerable extent: 40 cm. in the six-weeks-old pig, and between 30 cm. and 40 cm. in the sheep. In a young goat two or three weeks old he found the glands to extend only 4 cm. into the duodenum. For the ox he gives no measurements.

In my studies the Artiodactyla are represented by the sheep and pig, which are especially interesting because they represent the two extremes of specialization of the stomach of the recent Artiodactyla, and because they present differences in the relation of the pyloric glands to the glands of Brunner which may be compared to those shown by the Rodentia with simple stomachs and complex stomachs respectively. In the pig, in which the stomach presents the simplest form found in the group, the two kinds of glands resemble one another very closely; in the sheep, with a highly specialized stomach, the two kinds of glands are very different. They are further of interest because of Kuczynski's failure to stain the secretion by any of the methods he employed.

As far as the arrangement and topography of the glands are concerned, Hock's excellent description leaves little to be desired. In the pig, according to him, the glands of Brunner are the direct continuation of the enormously developed pyloric glands, which at the summit of the pyloric sphincter begin to divide more freely and extend more deeply, forming new lobules which completely fill the tunica mucosa and open by short tortuous ducts into the foveolæ gastricae. Presently they exceed the limits of the mucosa and extend over into and fill the submucosa. For about $1\frac{1}{2}$ cm., according to Hock, the lamina muscularis mucosæ is not present as a distinct layer, but the fibers are dispersed among the lobules of the glands, to the muscular investment of which the pyloric sphincter also contributes fibers. For a distance of 20 cm. from the pylorus the glands are described as forming a compact mass, completely filling the submucosa. He finds that the ducts at the beginning of the zone discharge into the gastric foveolæ; beyond the point of the first appearance of the glandulæ

intestinales of Lieberkühn, they empty almost exclusively into the latter. A few independent ducts, however, are present.

The large glandular tubules are composed of large cylindrical cells, 18μ – 21μ in height, surrounding a very narrow lumen. The nucleus is flattened or crescentic and placed in the base of the cell. The body of the cell exhibits a faintly staining network containing the secretion. Kuczynski (1890) was unable to obtain any specific staining of this secretion by the methods he employed. He was also unsuccessful in attempting to stain the secretion of the similar cells of the pyloric glands, although he remarks that the pyloric glands near the fundus zone stain with Victoria blue. He concluded that if they contain any mucin it was not their exclusive constituent. By the technique recommended at the beginning of this article, I have succeeded in staining the secretion of both the pyloric glands and the glands of Brunner intensely in muchæmatein and in mucicarmin. When so stained the meshes of the cytoplasmic network of the body of the cell are found to be filled with a compact mass of small granules. Staining in indulin-eosin-aurantia mixture also gave successful results. There is, therefore, no adequate reason for supposing that the cells of the glands of Brunner of the pig are essentially different in function from those of other mammals.

In the sheep the differences between the glands of Brunner and the pyloric glands are very striking, and I have found that even with the solutions which I have employed with success on other mammals the secretion of the Brunner's glands stains with difficulty.

The pyloric glands of the sheep are simple tubes composed of somewhat narrow triangular cells 12.5μ to 17μ in height with nuclei round, oval, or crescentic according to the shape and secretory condition of the cell. Around the nucleus is a very small amount of finely reticular cytoplasm. The body of the cell is transparent, and finely reticular. The secretion forms a continuous mass, and stains readily and deeply in muchæmatein and mucicarmin.

The glands of Brunner are exceedingly large tubules, with wide lumina surrounded by cylindrical cells 16μ to 22μ in height. The nucleus in these cells is spherical or oval, slightly cupped on the side directed toward the lumen, and located in the proximal end of the cell. The whole of the cell between the nucleus and the lumen is filled with secretion which is distinctly divided into a proximal and distal mass in many of the cells. The secretion stains in muchæmatein, but much more slowly, and less intensely, than that of the pyloric glands. The differences in shape and character of the tubules and cells of the glands of Brunner are particularly obvious opposite the sphincter pylori where glands of Brunner occur in the mucous membrane side by side with the pyloric glands. Very striking at this point is the contrast, in muchæmatein preparations, between the deeply stained narrow tubules of the pyloric glands and the faintly stained wide tubules of the glands of Brunner. The difference in staining capacity does not, as will be pointed out later, imply a difference in the amount of mucin secreted by the cells.

VII. THE GLANDS OF BRUNNER OF MAN

The material for this study consisted of the duodenum and a portion of the jejunum of an executed criminal, a young man about thirty years of age. The material was obtained about forty-five minutes after death and, although the epithelium of the free surface and portions of the villi were lost in places, proved to be in other respects excellently preserved. A strip from end to end of the duodenum was fixed in alcoholic sublimate bichromate mixture and the rest in 70 per cent. alcohol. For comparison a second duodenum obtained for the writer from the body of a woman seventy years of age, and fixed in alcoholic sublimate bichromate, was studied. In the latter marked cell-atrophy was exhibited by the glands of Brunner, but some interesting facts were obtained as to the mode of accumulation of the secretion in the cell.

The observations of the writer confirm in the main those of Renaut (1879), Schaffer (1891), and Castellant (1898) as to the distribution of the glands of Brunner in the intestine of man.

The glands make their appearance in the mucosa and submucosa, opposite the summit of the pyloric sphincter, at the point where the first intestinal gland of Lieberkühn appears. There is for a short distance, about 2.5 mm. in my material, a slight mingling of intestinal and gastric glands as observed by Böhm and von Davidoff (1895), and confirmed by Castellant (1898). In this region, however, only a few pyloric glands are visible, and in some of these goblet cells occur among the gastric epithelial cells.

The glands of Brunner located in the mucous membrane at this point form groups of radiating, slightly wavy, branched tubules, clustered around the base of a gland of Lieberkühn, or of a foveola gastrica into which they open. They are not in this region clearly marked off into lobules, and are a direct continuation of the pyloric glands which, as many writers have pointed out, exhibit a tendency to richer branching near the beginning of the duodenum.

In the pars superior of the duodenum from the first individual the surface of the mucosa presented a somewhat mammillated appearance, owing to the occurrence in it of many large solitary follicles (*noduli lymphatici solitarii*). In sections these solitary follicles form interruptions at regular intervals in the continuity of the glandular elements of the mucous membrane, as may be seen in Plate XXIV, Figs. 14 and 15. This fact gives in sections an appearance of regular grouping of the glands located in the tela submucosa as well as of those in the mucous membrane, because, obviously, the ducts of the former must open to the surface between the solitary follicles and the lobules of the glands must be arranged in a radial fashion around the ducts. This appearance is well illustrated in Fig. 15, where the lobules of the group in the submucosa spread out in a fan-like fashion from the point in the mucosa where their terminal ducts are located.

In the pars superior duodeni the glands of Brunner, as described by Renaut (1879) and confirmed by Schaffer (1891) and Castellant (1898), form two groups,

one located in the mucous membrane, the other in the submucosa. The former, except for the interruptions caused by the lymphatic nodules, form an almost continuous layer occupying exclusively the outer half of the mucous membrane, the inner half being occupied by the glands of Lieberkühn and the villi. The lobulation is not very striking, although, as shown in Fig. 14, the groups of glands which empty into a single duct may be easily recognized. These groups are somewhat elliptical or triangular in section, the long axes being perpendicular to the surface of the mucous membrane. From the inner end of each group one, two, or more ducts emerge, which either join to form a single duct, or open separately into the same gland of Lieberkühn or into side branches of the latter.

Each group is composed of a cluster of tubules, which are the primary branches of the duct, and of the numerous ramifications of the latter. The most prevalent mode of branching is as follows: the duct divides near its origin into a tassel-like group of wavy tubules which pass outward in the direction of the lamina muscularis mucosæ. Each of these tubules gives off on all sides radial descending branches, which in their turn, after a very short course, subdivide and terminate either as short tubules of the same diameter as the parent tubules, or as slightly expanded, elongated, pear-shaped acini. Some of the groups extend through the interrupted muscularis mucosæ to form lobules or groups of lobules in the inner layer of the tela submucosa.

The group of glands in the submucosa is composed of elliptical and fusiform lobules of small size, placed with their long axes nearly parallel to the free surface of the duodenum. These lobules are confined to the inner layer of the tela submucosa, there being usually an outer layer nearest the tunica muscularis free from glands and containing aggregations of adipose tissue. The lobules are not all independent of one another, several being often strung out along the same tortuous duct. Again, many of the larger lobules are subdivided into marginal lappets which represent the groups of branches clustered around each of the radial branches of the principal duct of the lobule.

The lobules of the submucous group of glands are derived from three sources. Some consist of the continuation into the submucosa of groups of tubules, the main bulk of which is located in the mucous membrane. In a second series a tubule, after giving off a number of branches in the mucous membrane, passes through an aperture in the lamina muscularis mucosæ, and gives rise to one or several lobules in the submucosa. In the third series a duct of variable size passes directly from the bottom or side of a gland of Lieberkühn to the submucosa, where it terminates in a lobule or a series of lobules. In the pars superior duodeni of the cases examined the second, in the pars horizontalis and pars ascendens of the organ the third, was the prevailing type.

The point of junction with the gland of Lieberkühn is a variable one, but I have not observed, in either of the two duodena examined, a single instance of the duct reaching the free surface independently of the intestinal glands, although that such cases do occur, is shown by the observations of Schaffer and Castellani.

As regards the distribution of the glands of Brunner in man, the observations of

the writer confirm the description given by Castellant (1898), except with respect to their downward extent. In this case the glands extend only within about 3.5 cm. of the duodenojejunal flexure; in Castellant's case isolated lobules were found in the upper part of the jejunum.

From the beginning of the pars descendens duodeni downward the glands are progressively reduced in bulk and tend, as Castellant observed, to become located in the plicæ circulares (Kerkringi), although not wholly confined to the latter. In the lower part of the duodenum they are reduced to scattered small lobules. Throughout, however, there are tubules in the tunica mucosa as well as in the submucosa, although the latter predominate. Castellant remarks concerning these tubules of the mucous membrane: "Elles cessent même presque complètement au niveau de l'union de la première portion du duodénum avec la seconde; on n'en retrouve plus qu'accidentellement au delà"—indicating that he observed them in the lower portion of the duodenum, but in smaller numbers than in the material described above.

In each of the lobules of the submucous group may be distinguished a central tubule which is the duct of the lobule. From this duct, which may be extremely tortuous in its course, come off numerous side branches of various lengths and complexity of secondary branching. After passing through one lobule, a duct may enter a second and a third, branching in each in a similar way; or some of the side branches may pass out and form the central duct of accessory lobules. Often the central duct of a lobule may be locally enlarged, as may be seen in some parts of Fig. 15.

The ducts pass through openings in the muscularis mucosæ sometimes singly, more often in groups, and empty into the bottoms, sides, or branches of the glands of Lieberkühn. In the submucous as in the mucous group, the terminal branches of the secreting tubules are short tubules of the same size as the main branches, or elongated, pear-shaped acini. In both cases they are formed of cells similar to those forming the ducts and their various branches.

All the tubules of the glands of Brunner of man are provided with a delicate basement membrane composed of reticulum. The ducts and tubules are formed of somewhat rectangular epithelial cells, 15μ to 21μ in height, uniform in type throughout the gland, but with some differences of structural details in different parts. In the terminal tubules in the material from the young subject, the cells are of the type represented in Plate XXII, Figs. 11 and 12. The nucleus is crescentic or flattened in form, and is located in the outer end of the cell. The body of the cell contains a network composed of extremely fine cytoplasmic fibrils forming large meshes in which the secretion of the cell is contained. In the center of the cell the cytoplasm forms a network of smaller meshes and coarser trabeculae corresponding to the band of cytoplasm separating the two masses of secretion in the cells of the opossum, cat, mink, etc.

As may be seen from Figs. 14 and 15, which are half-tone reproductions of photomicrographs of specimens stained in strong muchæmatein, the glands of Brunner stain even more intensely in this solution than the goblet cells of the

intestinal glands. In strong mucicarmine a similar result is obtained. The appearance of the stored-up secretion when stained in muchæmatein depends on the mode of fixation and subsequent treatment. In the material fixed in alcoholic bichromate sublimate, imbedded in celloidin, sectioned and stained, without passing through water, the secretion is in the form of minute granules of smaller size and less closely packed than those in cells from the glands of Brunner of the opossum. In sections cut in paraffin, fastened to the slide by the water method, and stained with muchæmatein, the secretion presents itself in the cell in the form of a coarse-meshed network. A result similar to the latter is obtained with material fixed in 70 per cent. alcohol, except that the meshes of the mucin network are much larger and are formed of thicker trabeculae.

In the ducts the cells are similar to those in the tubules and acini; indeed, many of the ducts of the small lobules are indistinguishable from the other tubules forming the lobules except by the method of tracing them out in serial sections. In the larger ducts, however, and particularly in locally dilated portions of them, the cells, while similar to those of the acini, tend to be more protoplasmic in nature. In such cells the division of the secretion into two masses and the transverse band of cytoplasm separating them are particularly obvious, and the cell presents a structure exactly comparable to that shown in Fig. 2, which is taken from the cells of the opossum. Zimmermann (1898) described and figured this condition in the cells of the glands of Brunner of man, but did not recognize the fact that the outer clear zone near the nucleus represented a second accumulation of secretion in that part of the cell.

In passing from a duct to a side branch of it, there is a gradual transition from this more cytoplasmic type of cell with spherical nucleus and two distinct masses of secretion to the secretion-filled cell with a single continuous mass and crescentic nucleus.

A similar transition occurs in the tubules of the glands located in the mucosa. A small gland from this source, together with a portion of the gland of Lieberkühn into which it opens, is shown in Fig. 12. In the gland of Lieberkühn in this figure the three typical elements—cylindrical cells, goblet cells, and one Paneth cell—are seen. The change to the epithelium of Brunner's gland, as indicated by Schaffer (1898), is abrupt, *i. e.*, there are no intermediate stages between it and the intestinal epithelial elements. In the Brunner's gland, however, a gradual transition is to be observed from the cell with a narrow band of secretion along the lumen, a large mass of cytoplasm, and a spherical nucleus, to the secretion-filled cell with a crescentic, basally situated nucleus. In some of the latter the remains of the band of cytoplasm separating the two primary masses of secretion may be clearly seen.

A point of considerable interest is the occurrence in the human glands of Brunner of a very small number of parietal cells exactly similar to those seen in the gastric glands. These cells occur in very small numbers, but their structure is so characteristic that there can be no doubt of the correctness of their identification. They

contain distinct intracellular ducts, and exhibit the three characteristic zones of structures described by Zimmermann (1898) for the parietal cells of the stomach. As in the latter the nuclei may be multiple.

The cells of the pyloric glands in man resemble very closely in size and structure those of the glands of Brunner. In general, in the former the subdivision of the secretion into two masses is more obvious and the proximal mass contains coarser cytoplasmic trabeculæ.

The cells of the glands of Brunner of the old subject were, as has already been remarked, considerably atrophied. They measured 12μ to 14μ in height. The lumen showed a corresponding enlargement. In most of the tubules the secretion stainable in strong muchæmatein was confined to a narrow zone less than 2μ in width along the lumen. The rest of the cell was occupied by a continuous mass of cytoplasm containing the oval nucleus. In some of the cells, however, a second small proximal mass of secretion occurred in the midst of this cytoplasm between the nucleus and the narrow distal mass, and in a few tubules the two had become confluent, forming a single large mass of secretion, filling all of the space between the now flattened nucleus and the lumen.

VIII. DISCUSSION OF RESULTS

The results of this investigation show that there is a remarkable uniformity in the nature and structure of the glands of Brunner of many mammals. In eighteen out of the nineteen genera examined the glands are of the pure mucous type. This conclusion is based on the structure of the cells of the glands, and on their staining and microchemical properties. The evidence, which is partly negative, partly positive, may be briefly summed up as follows:

The cells of the glands, when examined fresh in serum or normal salt solution, do not show easily visible secretion-granules. The granules (droplets?) of secretion like those of known mucous glands correspond so closely in refractive power with the mounting media that they are almost invisible.

The cells when fixed and stained do not contain basal filaments (prozymogen), and the microchemical test for organic iron indicates the presence of only a relatively small amount of cytoplasmic nucleoproteid. Serous cells from other sources, on the other hand, show the presence of a large amount of cytoplasmic nucleoproteid, either in the form of the so-called basal filaments (composed largely of the nucleoproteid prozymogen) or in the form of prozymogen diffused in the basal cytoplasm.

The granules of zymogen in the pancreatic and gastric ferment-secreting cells stain strongly in iron hæmatoxylin. The secretion of the cells of the glands of Brunner remain colorless in this stain.

A more positive and selective stain for the granules of zymogen of the stomach and pancreas, Reinke's neutral gentian, as modified by the writer (1900), also gives negative results with the cells of the glands of Brunner.

The secretion granules of the cells of the glands of Brunner give no reaction when tested by Macallum's method for the microchemical detection of organic phosphorus. The progress of this reaction is easily controlled, in the case of the glands of Brunner, by observing the effect on the granules in adherent sections of the pancreas, which give a positive reaction for organic phosphorus after treatment for two hours with nitric acid ammonium molybdate. The results with this test in the glands of Brunner, with the exception of the dark tubules of the glands in the rabbit, are absolutely negative.

Not only do the cells of Brunner not contain the chemical substances which it is possible to recognize in the serous cell either by examining the fresh cell or by employing staining and microchemical methods, but, on the contrary, there is positive evidence that they contain something else, which we have good reason to believe is mucin.

The secretion contained in the cells of the glands of Brunner stains with uniform facility, if certain precautions are taken, in Mayer's alcoholic muchæmatein and mucicarmine. With muchæmatein the precautions necessary to success are as follows:

If the dilute solution recommended by Mayer is employed, the sections cut in paraffin should be transferred, *without attaching them to slides*, to benzole, thence to absolute alcohol, thence directly to the staining solution. After five minutes in the latter, they are washed with 70 per cent. alcohol, dehydrated, cleared, and mounted.

A stronger solution (having the following formula: hæmatein, 1 g., aluminium chloride, 0.5 g., 70 per cent. alcohol, 100 c.c.) gives better results, and can be used on sections fastened to the slides or on celloidin sections with certainty of speedy and satisfactory results. The degree of acidity of this solution is of some importance. The writer is in the habit of reducing the acidity by diluting his alcohol with tap water containing calcium bicarbonate. After the solution so prepared has stood for a week, it is tested on a section. If the resulting stain is slightly diffuse, a 10 per cent. solution of nitric acid is added, a drop at a time, the staining properties being tested on a section after the addition of each drop of acid. This is kept up until the correct reaction is obtained. The solution so prepared is employed as follows:

The sections cut in paraffin and fastened to the slide by the albumen or by the water method are treated with benzole followed by absolute alcohol. The slide having been placed on the stage of the microscope, a drop of the staining solution is applied to the section, and the latter is watched under the microscope until a proper depth of color is obtained in the secretion within the cells. It is then rapidly washed in 70 per cent. alcohol, dehydrated, cleared in benzole, and mounted in benzol balsam.

If the staining is prolonged and the solution is not renewed from time to time, the sections after attaining a maximum depth of color will slowly fade out again, probably owing to reduction in the acidity of the solution by the absorption of ammonia from the atmosphere. Sections should not be washed in water after staining, as this procedure completely removes the stain from the mucous cells.

This stronger muchæmatein solution *stains deep blue* the secretion contained in

cells from the following sources: mucons cells from the submaxillary, sublingual, lingual, palatine, tracheal, and œsophageal glands; the gastric epithelial cells; the cells of the cardiac glands of the stomach; the cells of the pyloric glands; the neck chief cells of the fundus glands of the stomach; goblet cells; and the cells of the glands of Brunner (except the dark tubules of the rabbit's glands). *It does not stain* the secretion in cells from the following sources; demilune cells of the salivary glands; the cells of the parotid gland; the serous cells of the submaxillary or sublingual glands; the serous portions of the palatine glands and tracheal glands; nor the ferment-forming cells of the pancreas and of the fundus glands of the stomach.

An idea of the intensity of the resulting color got by this method may be obtained from Figs. 14 and 15, which are half-tone reproductions of photomicrographs of sections of the human glands of Brunner stained in stronger muchæmatein. An equally strong stain was obtained in the glands of Brunner of all the animals examined, with the single exception of the sheep, in which the secretion stained positively, but more slowly and with less intensity.

For mucicarmine the conditions of success are that the solution be employed undiluted in the form of Mayer's stock solution, and that it be freshly prepared. In the writer's hands the solution, after twenty-four to forty-eight hours, refuses to stain and cannot be filtered. The solution is to be applied in exactly the same way as muchæmatein and gives similar results, both on the glands of Brunner and on the other glands mentioned. In view of the results in the several glands of known character which are enumerated above, we are justified in concluding that the solutions employed as recommended do stain mucous cells and do not stain serous (zymogenic) cells.

In view of Mayer's observation that the clear cells of the submaxillary gland of the hedgehog, which do not secrete mucin, stain with muchæmatein, some conservatism must, however, be exercised in interpreting the results. It is obvious that no absolute proof of the mucous character of the glands of Brunner can be brought forward until a positive microchemical test for the various mucins is devised, or until some one undertakes and completes the laborious task of isolating the lobules of Brunner's glands carefully by dissection and studying them by the ordinary macrochemical methods. The mucous nature of the glands is, however, supported by the recent work of Ponomareff (1902) in Pawlow's laboratory, who isolated a portion of the duodenum by Thiry's method as modified by Pawlow. The juice obtained was colorless, thick, and very viscid.

Further evidence is, however, afforded by the tests applied to determine the solubility of the secretion in various solutions. This is accomplished by using muchæmatein as an indicator. The sections are fastened to the slide by the water method, and are placed in the solution to be tested. From time to time a section is taken out, washed thoroughly, and stained by muchæmatein. By this means it has been determined that the contents of the cells of the glands of Brunner of the opossum and of man are soluble in weak alkaline solution, insoluble in 5 per cent. solution of hydro-

chloric acid and in artificial gastric juice containing 0.2 per cent. of hydrochloric acid.

The structure of the cells also supports the conclusion that they are mucous cells.

The cells of the glands of Brunner differ in structure according to the physiological phase in which they happen to be when examined. Three well-defined stages may be discerned: In the condition of maximum loading the cells are large and transparent. They contain a flattened or crescentic nucleus, located in the base of the cell, surrounded by a small quantity of finely reticular cytoplasm. The body of the cell is clear and shows a coarse network of cytoplasmic trabeculæ in the meshes of which the secretion is lodged.

In the intermediate condition, the nucleus is more oval in outline, the basal cytoplasm is greater in amount and the body of the cell exhibits two distinct secretory zones. In the proximal zone the granules of secretion are separated from one another by cytoplasmic trabeculæ coarser than those of the distal zone. (See Fig. 2.)

In the discharged condition the nucleus is spherical or oval and nearer the center of the cell. The basal cytoplasm is increased in amount. The secretion may be confined to a mass on the free border of the cell, or there may be two masses, a dense one on the free border (referred to in the specific descriptions as the distal mass) and a less dense one (the proximal mass) composed of smaller granules, in the interior of the cell.

The two latter conditions may be reached either as stages in the discharge of the cell, or in inverse order, as stages in its recovery during a period of rest after discharge of its secretion.

In the writer's opinion, the obvious subdivision of the secretion into two masses is due to the fact that the new secretion is formed in the neighborhood of the nucleus in the interior of the cell. This may be due, as suggested above, to the action of enzymes produced by the nucleus, or it may be due to the effect of the presence (of which the writer has not yet been able to obtain evidence) in these cells of structures similar to the so-called trophospongium observed by Holmgren (1902) in various epithelial cells.

A similar secretory mechanism has been shown to exist in the various mucous salivary glands by the studies of Maximow (1901) and Kolossow (1903). Both of these writers have noticed the obvious division of the secretory portion of mucous cells into two zones, and Maximow has also observed the new formation of secretion granules in the neighborhood of the nucleus. I have (1898, 1902) demonstrated similar conditions in the cells of the palatine glands, pyloric glands, cardiac glands, and in the mucous neck chief cells of the fundus glands of mammals. Krause (1895) has also described the formation of new secretion near the nucleus in the mucous cells of the retrolingual glands of Erinaceus.

The conclusion that the glands of Brunner are mucous glands is concurred in by a number of writers mentioned in the introductory paragraphs. Castellant (1898), Kuczynski (1890), and Schaffer (1891) came to a similar conclusion, with some reservations. The two former, using various synthetic stains and ordinary solutions of hæmatoxylin, have observed that the secretion of the glands of Brunner of different

mammals, and indeed of different tubules of the same animal, stain with different degrees of facility. They conclude that the depth of staining indicates the amount of mucin present in the cell, and that some cells contained a great deal of mucin, others a little, and still others none at all. The uniformly intense stain obtained by the writer by means of his muchæmatein technique shows that this conclusion is not justified. The cells are to all appearance in all mammals equally engaged in mucin secretion.

Does the different capacity for staining in these synthetic dyes indicate, as Schaffer (1891) thought, a difference in the nature of the mucins formed? It is, of course, possible that this is the case. We already know many different mucins, and we have reason to suspect, as Huppert (1896) has suggested, that there is a great number of different glycoproteids. Although it is possible that different mucins are secreted by the glands of Brunner in different mammals, it does not appear to me to be necessary to assume that this is the case in order to explain the different staining properties.

It is known that the mucous cells do not store their secretion as mucin, but as a substance (mucigen) which may readily be transformed into mucin. It is not possible definitely to identify the granules visible in the cell as mucigen, but they are probably composed of one of the antecedents of mucin, and not of mucin itself. It is, moreover, probable that the transformation of the substances received by the cell into mucin is not accomplished by one or even two steps, but that there are many stages in this chemical process. Furthermore, it is a well-known fact that similar cells from different sources, engaged in the formation of the same product, may store it in the form of different antecedent substances. For example, the chief cells of the glands of the gastric fundus of the rabbit in the resting condition are filled with zymogen granules and contain little prozymogen. The similar cells of the glands along the greater curvature contain few granules, but a great deal of prozymogen. These cells differ, as Langley (1882) pointed out, in secretory equilibrium.

It is conceivable that mucous cells similarly differ in secretory equilibrium, and that, while the ultimate product of their secretory activity may be the same substance, they contain the antecedent substances in varying proportions. Some such explanation as this must be resorted to, in order to explain why similar cells of the same tubule differ in their staining capacity.

The glands of Brunner of the rabbit are mixed glands. The bulk of the cells composing the tubules are mucous cells similar in all important respects to those in other mammals. In many cases the dark cells forming the terminal acini or tubules are specifically different from the mucous cells. There are no intermediate stages, and these cells do not under any conditions contain mucin or its stainable antecedents. They contain zymogen granules, easily visible (as Schwalbe first pointed out) in the fresh cell, and prozymogen. By the microchemical reactions for iron and phosphorus these elements may be demonstrated to be fundamentally different from the contents of the mucous cells.

As regards the similarity of the glands of Brunner to the pyloric glands of the stomach, it may be said that in nearly all cases examined slight differences in structure could be discerned. I do not, however, regard these differences as of fundamental importance. Certainly they are primitively cells of the same type. An interesting fact is that the greatest differences between the cells of the two sorts of glands were found, in the series of animals studied by the writer, in those animals in which the stomach was highly specialized. This fact can be explained by the assumption that the two groups of glands were primitively similar, and that their great dissimilarity in the case of animals with specialized stomachs is due to the fact that the pyloric glands have also been modified in the course of this specialization. The writer, however, hesitates to generalize in this respect until a much larger series of animals has been studied by the methods employed in this research.

The question of the phylogeny of the glands of Brunner is an exceedingly difficult one to discuss. Up to the present the only clear-cut theory of their origin advanced is that of Oppel (1897). This author expresses his views as follows:

Bei zahlreichen niederen Wirbeltieren finden sich Spuren einer Tendenz der Pylorusdrüsen, sich über den Sphinkter hinaus auszubreiten, so z. B. bei Urodelen, wo eine scharfe Grenze zwischen den letzten Pylorusdrüsen und den Darmdrüsen überhaupt schwer zu ziehen ist. Die letzten Pylorusdrüsen zeigen ferner bei manchen Reptilien und Vögeln an ihren unteren Enden die Tendenz, sich stärker zu entwickeln, eine Tendenz, die auch noch bei Säugern zum Ausdruck kommt. Verbinden wir beides, so werden wir leicht den Vorgang der Entstehung der Brunnerschen Drüsen so deuten können, dass die Drüsen der Pylorusdrüsenzzone über den Sphinkter hinauswachsend und zu einer excessiven Entwicklung gelangend, die Muscularis mucosae durchbrechen und so zu Brunnerschen Drüsen werden.

The attractiveness of this hypothesis becomes apparent when we examine such a case as that of the opossum, in which the glands all open on circumscribed areas of the intestine, covered by gastric epithelium. The facts in favor of the hypothesis are briefly: the contiguity of the glands of Brunner to the pyloric glands in the less specialized mammals; and the great similarity of the cellular components. As regards the similarity of the cells, however, it must be remembered that the cells of the glands of Brunner resemble just as strongly the mucous cells of many buccal, œsophageal, and tracheal glands. We are thus reduced to the fact of contiguity as an effective argument for the phylogenetic development of the pyloric glands into glands of Brunner. Furthermore, in accepting this hypothesis we must assume that the epithelium of the small intestine is specifically differentiated from that of the stomach, not only in the adult, but in the embryo at the time the glands of Brunner are formed; that is to say that at a time when no structural differences can be discerned between the cells of the gastric and intestinal epithelium the cells actually have a different developmental potential, those of the stomach having lost the power of developing into cells of the intestinal type and those of the intestine that of developing into cells of the gastric type. We must also assume that there is a mingling of this gastric hypoblast and intestinal hypoblast in the region of the formation of the glands of Brunner, because

ontogenetically the glands of Brunner do not develop as downgrowths of pyloric glands, but develop simultaneously as independent elements in the duodenum. Furthermore, if we assume this extremely early specification of the respective epithelial elements of the stomach and intestine, how are we to explain the occurrence of glands of Brunner in the horse at a point seven meters from the pylorus, and the relatively great extent of the glands in the rabbit, sheep, pig, and man? It might, of course, be urged that it is possible that in the rapid growth of the midgut, gastric epithelial elements may be carried a considerable distance from the pylorus and there serve as foci for the development of glands of Brunner. A similar argument might be employed to explain the occurrence of characteristic intestinal epithelium in the stomach, as observed by Schaffer (1897), Boeckelman (1902), and Hári (1901). Such arguments are unanswerable because they do not admit of proof or disproof.

It does not seem possible to me to reconcile the facts of the distribution of the glands of Brunner and of their ontogenetic development with Oppel's theory that they are developed as a further downward growth of the pyloric glands into the intestine. For the present it would seem to be more probable that the glands of Brunner are cænogenetic structures developed in mammals from the hypoblast of the midgut. The occurrence of serous tubules in the glands of Brunner of the rabbit is evidence of a new functional need in the intestine.

Oppel (1899) has, however, promised further explanations of his theory to adapt it to the facts of distribution, and in the meantime these may be awaited with interest.

In conclusion I should like to emphasize the fact that I do not regard the evidence brought forward in this investigation to show that the glands of Brunner are mucous glands as at all excluding the possibility that they also form small quantities of digestive ferments. The latter, however, if they are formed, are not present in sufficient quantities to appear in the cells as definite formed elements recognizable by the microscopic or microchemical means at our disposal.

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EXPLANATION OF FIGURES

For the drawings, of which Figs. 1-11 and Fig. 13 are reproductions the author is indebted to the skill of Mr. Leonard H. Wilder. All drawings are from camera lucida outlines.

PLATE XIX

FIG. 1.—Section including a portion of the pars pylorica ventriculi and the upper end of the duodenum of the opossum, showing the peculiar defect of the tunica mucosa on which the glands of Brunner open. $\times 40$.

PLATE XX

FIG. 2.—Transverse section of two tubules of the glands of Brunner of the opossum. Ferric alum hæmatoxylin. The drawing shows the structure of the cells composing these tubules in the condition of incomplete loading. The secretion is subdivided into a proximal and distal mass by a band of cytoplasm. Some basal cytoplasm still remains at the attached end of the cell and around the nucleus. $\times 1100$.

FIG. 3.—Transverse section of a tubule of the glands of Brunner of the opossum in the same physiological condition as those in Fig. 2, stained with stronger muchæmatein. The granular character of the secretion, its subdivision into two masses, and the clustering of the granules of the proximal mass around the oval or flattened nucleus are well shown. $\times 1100$.

FIG. 4.—Tubule of the gland of Brunner of the opossum in the condition of maximum loading; iron hæmatoxylin. The two masses of secretion are not to be recognized, the transverse band of cytoplasm separating them having disappeared; basal cytoplasm less than in Fig. 2; nucleus more flattened. $\times 1100$.

PLATE XXI

FIG. 5.—Portion of a lobule of Brunner's glands of the rabbit, stained in iron hæmatoxylin. The figure shows nine mucous tubules and one serous acinus in transverse section. In the former the subdivision of the secretion into two masses is indicated in most of the cells. $\times 500$.

FIG. 6.—The serous acinus shown in Fig. 5 more highly magnified. In each cell the outer zone filled with deeply staining prozymogen and the inner clear zone containing a few granules of zymogen are shown. Most of the zymogen granules which are present in the living cell have been lost in fixation. $\times 1300$.

FIG. 7.—Fresh serous acinus from the rabbit's glands of Brunner showing inner zone filled with zymogen granules. $\times 1300$.

FIG. 8.—A tubule of Brunner's glands of the opossum. The section has been treated with water and then stained with stronger muchæmatein. The granules visible in Fig. 3 have given place to a deeply stained coarse-meshed network. $\times 1100$.

PLATE XXII

FIG. 9.—Tubule of the glands of Brunner of the muskrat. Composed of large mucous cells with flattened nuclei. Transverse band of cytoplasm separating the contained secretion into two masses is readily visible. $\times 720$.

FIG. 10.—Portions of three pyloric glands of the muskrat. Note the smaller cells, larger nuclei, and more abundant cytoplasm as compared with Fig. 9. $\times 720$.

FIG. 11.—A small lobule from the glands of Brunner of man. $\times 190$.

FIG. 12.—A portion of a lobule of the glands of Brunner of man located in the tunica mucosa. The duct of the gland opens into a gland of Lieberkühn, where a sudden change in character of the cells takes place. The cells of the duct become richer in secretion the farther they are from the point of entrance into the intestinal gland, the nuclei at the same time becoming more flattened. In many of the cells may be seen the transverse band of cytoplasm separating the secretion into two masses. $\times 280$.

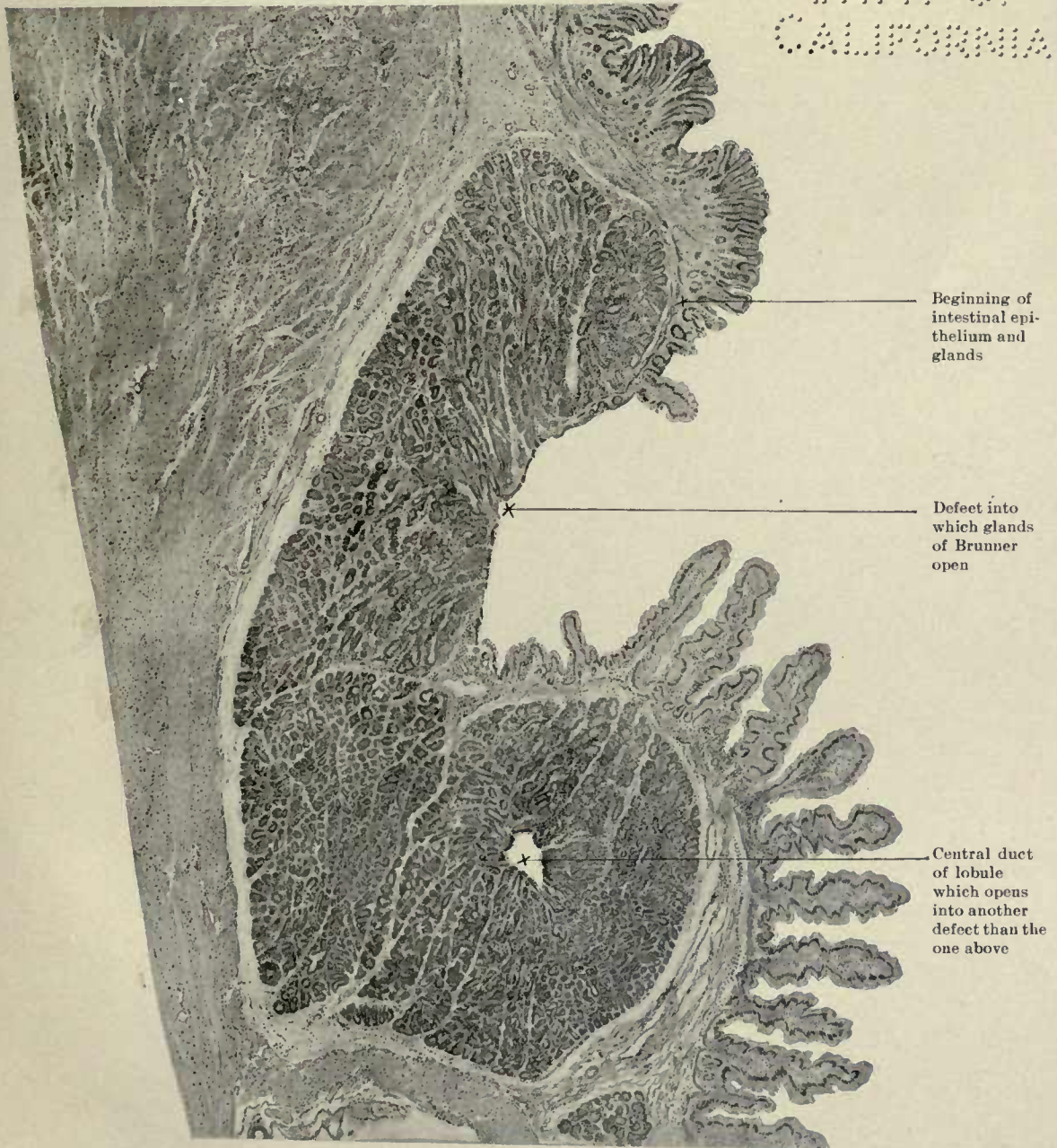
PLATE XXIII

FIG. 13.—Section of the duodenum of man. The superficial epithelium and portions of the villi in this section were unfortunately lost. The grouping of the glands of Brunner in the tunica mucosa and tela submucosa are shown, as well as their opening into the glandulæ intestinales. $\times 66$.

PLATE XXIV

FIGS. 14, 15.—Photomicrographs of section of the duodenum of man stained in stronger muchæmatein. These figures show well the topography of the glandular lobules as well as the intensity of the stain which their contained mucin takes in this solution. $\times 37$.

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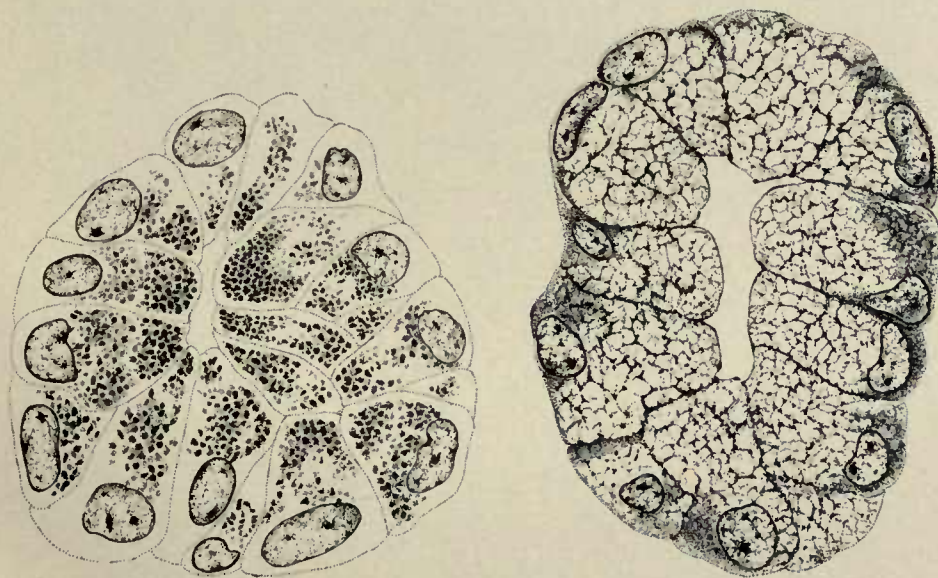


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FIG. 1



FIG. 2



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FIG. 3

FIG. 4



FIG. 5

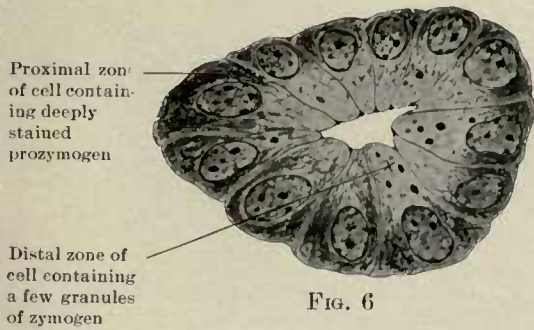


FIG. 6



FIG. 7

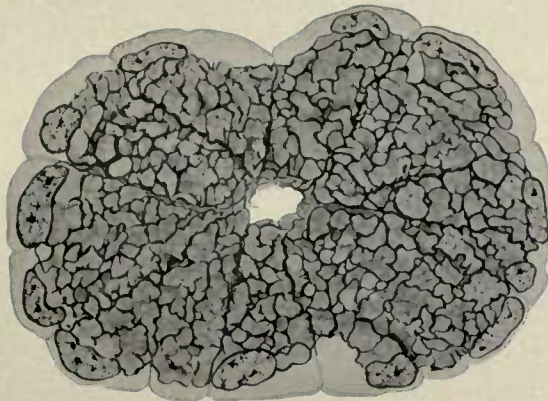


FIG. 8

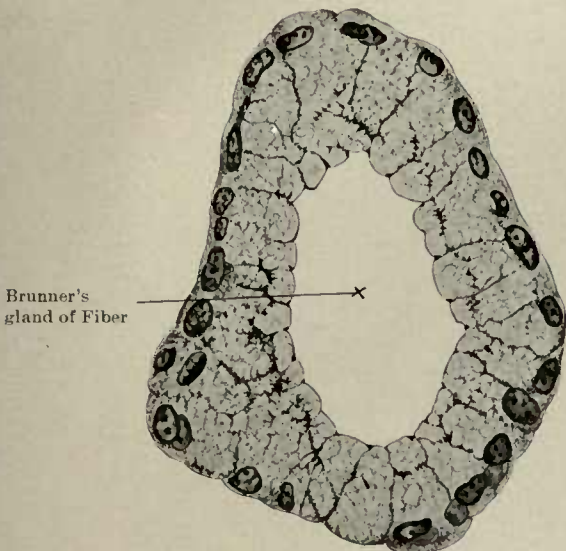


FIG. 9

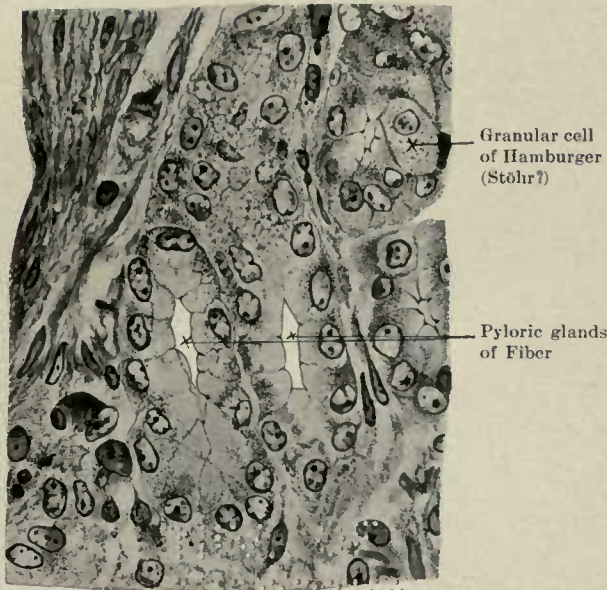


FIG. 10

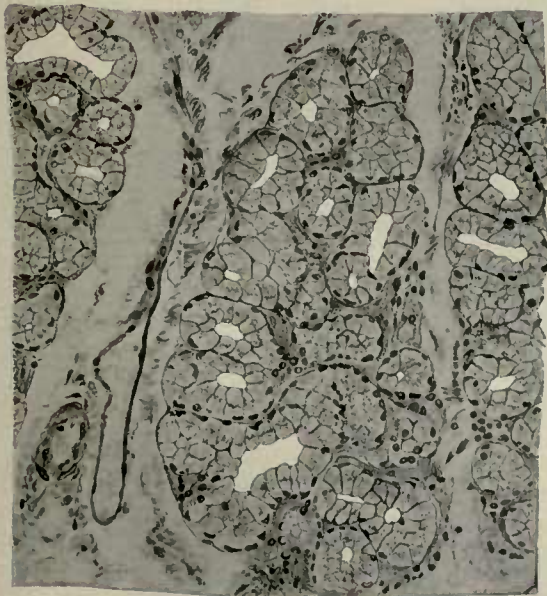


FIG. 11

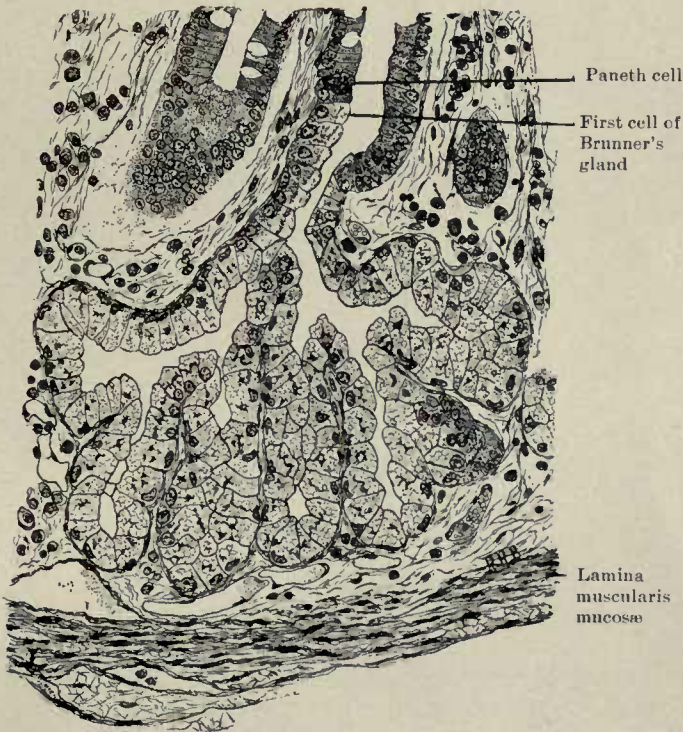
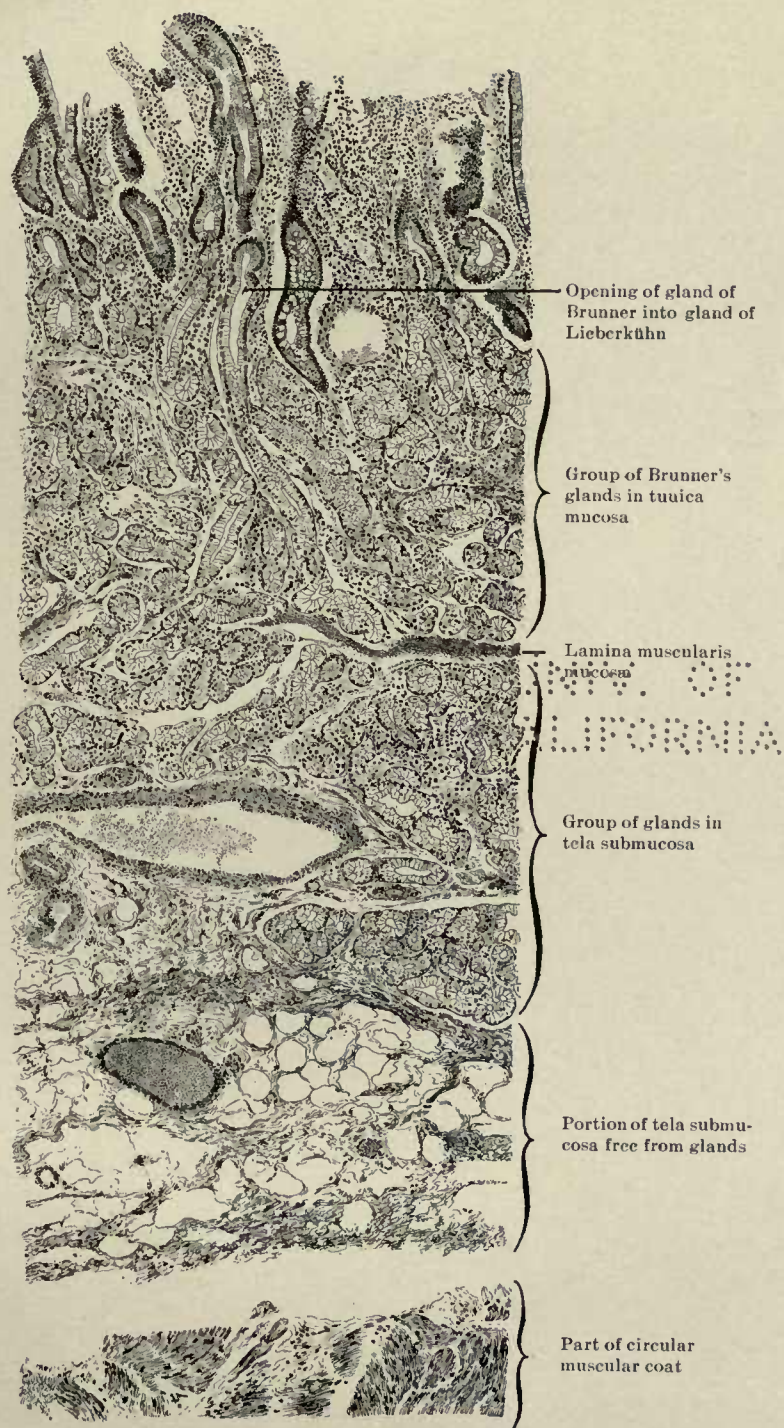
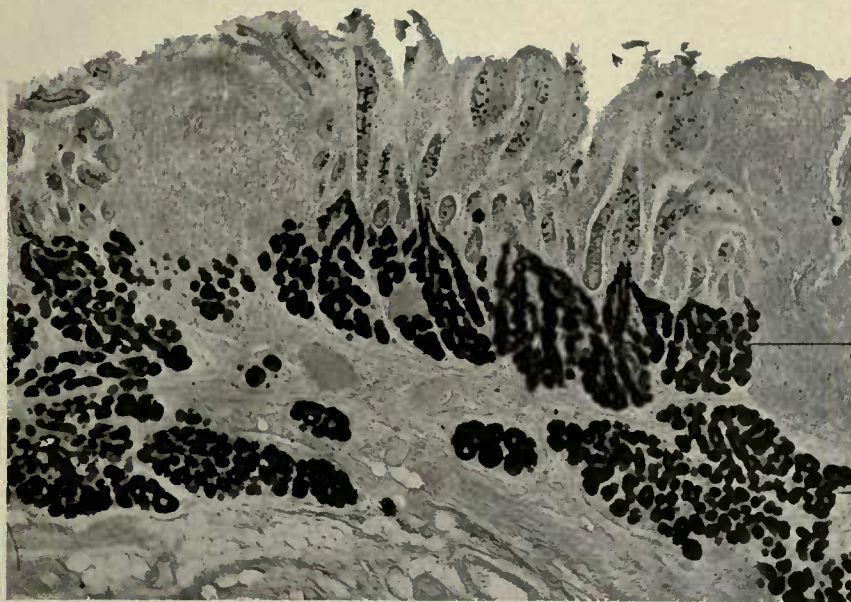


FIG. 12



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FIG. 13



Groups of glands in the tunica mucosa, opening into crypts of Lieberkühn

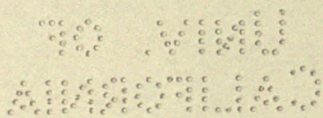
Submucous group of glands

FIG. 14



Dilated duct between lobules

Lobule showing central duct with radial branches



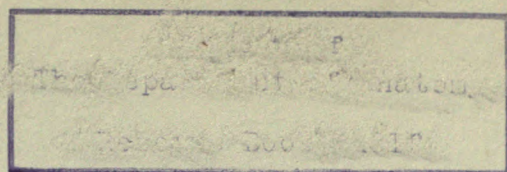
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